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ORIGINAL INVESTIGATION

Reciprocal responsiveness of nucleus accumbens shell and core dopamine to food- and drug-conditioned stimuli

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Abstract
Rationale Drugs of abuse and palatable food share the ability to stimulate dopamine (DA) transmission in the nucleus accumbens shell. However, while the stimulation of shell DA by food undergoes habituation, that by drugs of abuse does not.
Objective This study aims to directly compare the changes of extracellular DA, by microdialysis, in shell and core and prefrontal cortex (PFCX) in response to food- and drug-conditioned stimuli (CSs).
Methods Rats were trace-conditioned by Fonzies box (FB) or vanilla box (VB; CS), followed by food: Fonzies, intraoral chocolate solution (food-unconditioned stimulus (US)) and morphine (1.0 mg/Kg sc; drug US). Control (unconditioned) rats received standard food instead of Fonzies, tap water instead of chocolate, saline instead of morphine.
Results Food–CSs increased core but not shell DA, while drug–CSs did the opposite. Food and drug–CSs both increased PFCX DA. Exposure to food–CSs potentiated core and PFCX DA response to food while shell responsiveness was dependent upon the relative CS and US nature. If the CS was intrinsic to the food US (CS=FB/US=Fonzies) the response of shell DA to the US was abolished. If the CS was extrinsic to the food US (CS=FB/US=chocolate; CS=VB/US=Fonzies), shell DA increased in response to the US. Exposure to the drug–CS potentiated the DA response to the drug–US in the shell and in the PFCX, but not in the core.
Conclusion Drug–CSs differentially activate DA as compared to food–CSs in shell and core and differentially affect DA response to the US in these areas. These differences might be relevant for the role of DA in the mechanism of drug addiction.

Keywords Dopamine · Drug abuse · Food · Conditioning · Accumbens

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Introduction

Addictive drugs share with natural rewards the property of increasing DA transmission preferentially in the nucleus accumbens (NAc) shell (Pontieri et al. 1995, 1996; Tanda et al. 1997; Bassareo and Di Chiara 1997; Bassareo et al. 2003; Gambarana et al. 2003; Aragona et al. 2008). Activation of DA transmission by food rewards undergoes rapid habituation in the NAc shell but not in the core or in the prefrontal cortex (PFCX; Bassareo and Di Chiara 1997, 1999a and b; Rada et al. 2005; Danielli et al. 2009).These adaptive properties of food-induced stimulation of DA release in the NAc shell are consistent with a role of shell DA in Pavlovian incentive learning, i.e., in the acquisition of incentive properties by reward predictive stimuli (Di Chiara 2002; Spina et al. 2006; Fenu et al. 2006; Di Chiara and Bassareo 2007). It has been suggested that resistance to habituation of the ability of drugs of abuse to preferentially stimulate DA transmission in the NAc shell results in abnormal strengthening of stimulus–drug associations and acquisition of excessive motivational properties to discrete stimuli or contexts predictive of drug availability. This abnormal incentive learning process has been suggested to constitute the first stage of drug addiction (Di Chiara 1998). Consistent with this hypothesis is the observation that intermittent binge eating of sugar eliminates habituation and induces drug addiction like feeding (Avena et al. 2008).

Stimuli conditioned (CSs) by Pavlovian association with drugs of abuse are thought to play an important role in the acquisition, maintenance, and relapse of drug dependence and DA might be involved in these actions (Stewart et al. 1984; Robinson and Berridge 1993; Shaham et al. 2003; Everitt et al. 1999; Ciccocioppo et al. 2002; Volkow et al. 2003). Recently, we have shown that stimuli conditioned to morphine and nicotine stimulates DA transmission in the NAc shell and in the PFCX but not in the NAc core (Bassareo et al. 2007). As reviewed in the "Discussion" section, it is difficult to compare these results with those of other studies due to experimental (e.g., Pavlovian versus instrumental conditioning) and methodological differences (e.g., use of microdialysis versus voltammetry for estimating extracellular DA; Ito et al. 2000; Phillips et al. 2003; Roitman et al. 2004). On the other hand, even in the case of our microdialysis experiments, a direct comparison between the effect of food- and drug-conditioned stimuli on DA transmission in different DA terminal areas is made difficult by the fact that different experimental conditions were utilized for acquisition and scoring of responses conditioned by food and, respectively, by drug (Bassareo et al. 2007).

The goal of the present study was therefore twofold: first, to directly compare the impact of drug– and food–CSs on in vivo DA transmission by applying the same conditions and scoring utilized in our previous study on drug-conditioned stimuli (Bassareo et al. 2007); second, to investigate the mechanism of the inhibitory influence of exposure to food–CSs on the responsiveness of NAc shell DA to the food–US and to test the hypothesis that this inhibition is a case of the general phenomenon of habituation described by us (Bassareo and Di Chiara 1997), being related to generalization of the stimulus properties of the CS to the US due to sharing of some critical sensory properties of these stimuli.

In order to test this hypothesis, we studied the effect of either a new CS (vanilla box, VB) extrinsic to the US (Fonzies feeding) or the effect of the same CS utilized in previous studies (Fonzies Box, FB) but now associated to a different US (intraoral chocolate). For comparative purposes, we also studied the effect of a new CS (VB) conditioned to a drug (morphine). The effect on extracellular DA of CS presentation and food and morphine administration was studied in rats implanted with microdiaysis probes in the NAc shell and core and in the PFCX.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan Italy, Udine, Italy) weighing 200–250 g were housed in group of six per cage with standard food (MIL topi e ratti, GLP diets, Stefano Morini, S. Polo D'Enza, RE, Italy) and water ad libitum, for at least 1 week in the central animal room, under constant temperature (23 C°), humidity (60%), and a 12-h light/dark cycle (light from 8 a.m. to 8 p.m.).

All experimental procedures met the guidelines and protocols approved by the European Community (EEC Council 866609; DL 27.01.1992, No 116) and by the Ethical Commission for Animal Care and Use of the University of Cagliari.

Materials

Morphine hydrochloride (1 mg/Kg; S.A.L.A.R.S., Italy) was dissolved in saline and injected subcutaneously in a volume of 1 ml/Kg body weight.

Fonzies (KP Snack foods, Germany) is a snack food made of corn flour, hydrogenated vegetable fat, and cheese powder, provided with a distinct odor.

Chocolate solution was obtained using Nesquik Syrup (Nestlè, Austria) made of sugar, water, fat-reduced cocoa powder 10%, invert sugar syrup, dextrose, acidity regulator citric acid, salt, preservative (potassium sorbate), flavorings. The Nesquik syrup was diluted 1:1 with tap water.

The solution was administered through an oral catheter at a constant rate of 0.2 ml/min and the total amount drank was 1 ml.

Vanilla flavor solution was made by water, 1,2 propylenglicole, glycerol, vanilla flavor (Cameo, Italy).

Perforated cylindrical boxes were made of plastic and were 8 cm in height and 6 cm in diameter. Holes were very small. They were filled up with FB or with cotton wad soaked with 200 μl of vanilla flavor (VB) and were presented to the animals during training procedures and experiments.

Conditioning protocol

All training sessions were performed between 9.00 a.m. and 14.00 p.m. Rats were brought from the main animal room to a room under the same conditions of temperature, humidity, and light, and were transferred to smaller, individual cages (width, 23 cm; height, 16 cm; length, 38 cm) with standard food and water ad libitum. Fonzies- and chocolate-conditioned groups were presented with FB for 10 min; and then, after withdrawal of the FB, with Fonzies or were infused intraorally with chocolate solution. Fonzies- or morphine-conditioned groups were presented with VB for 10 min and then, after withdrawal of the VB, were given Fonzies to eat or were administered with morphine (1 mg/Kg s.c.). The control groups received standard food instead of Fonzies, intraoral tap water instead of chocolate and saline s.c. instead of morphine. These training sessions were repeated once every day for 3 days. On the fourth day, rats were brought to the surgery room

where they were anesthetized and implanted with microdialysis probes. Then rats were brought, while still anesthetized, to the experimental room and placed in large hemispheric bowls (Ø50 cm) with water and food ad libitum (see above). On the next day, water and food were removed and microdialysis and behavioral experiments were performed in the same bowls.

Probe and oral catheter preparation

Vertical microdialysis probes were prepared with AN69 fibers (Hospal Dasco, Bologna, Italy), according to the method of Di Chiara et al. (1993), modified by Tanda et al. (1996), with a dialysing portion of 1.5 mm for the NAc shell and core and of 3 mm for the PFCX.

Oral catheters were made of a 22-gauge stainless steel needle and of a polyethylene (PE) tubing (polyethylene tubing, Portex limited, Hythe, Kent, England; ID 0.58 mm and OD 0.96 mm). The 22-gauge stainless steel needle was cut on one side (length 2 cm), was blunted and inserted in the PE tubing which was ending with a perforated circular disk.

Surgery

All rats subjected to surgery session were anesthetized with 320 mg/Kg i.p. of chloral hydrate (Carlo Erba, Milano, Italy). Standard microdialysis probes were implanted in the NAc shell (A: 2.0, L: 1 from bregma, V: −7.8 from dura), NAc core (A: 1.4, L: 1.6 from bregma, V: −7.6 from dura) and PFCX (A: 3.7, L: 0.8 from bregma, V: −5.0 from dura) according to the atlas of Paxinos and Watson (1998).

One week before training sessions, rats that during training and experiment receive chocolate as US and their controls were anesthetized with 320 mg/Kg i.p. of chloral hydrate (Carlo Erba, Milano, Italy) and implanted with an oral catheter. The oral catheter was inserted at the level of the first molar, then the PE tubing passed along the skull and came out through the skin at the level of the ear where it was fixed using a cylindrical piece of rigid plastic filled up with ciano-acrylic glue (Attak, Henkel, Milano, Italy). This technique was simpler and less traumatic with respect to that previously described by us (Bassareo et al. 2003).

Analytical procedure

On the day following surgery, the probes were connected to an infusion pump and perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$) at a constant rate of 1 μl/min.

Dialysate samples (10 μl) were taken every 10 min and injected into a high-performance liquid chromatograph equipped with a reverse phase column (LC-18 DB, 15 cm, 5 μm particle size, Supelco) and a coulometric detector (ESA, Coulochem II, Bedford, MA, USA) to quantify DA. The first electrode of the detector was set at +175 mV (oxidation) and the second at −225 mV (reduction).

The composition of the mobile phase was: 50 mM NaH_2PO_4, 0.1 mM Na_2-EDTA, 0.5 mM n-octyl sodium sulfate, 15% (*v*/*v*) methanol, pH 5.5 (obtained adding Na_2HPO_4). The sensitivity of the assay for DA was 5 fmol/sample.

Every subject was tested only once during the experimental session.

Behavioral recording

Rats were videotaped during microdialysis experiments after FB or VB presentation. The following behavioral items were recorded: *orienting reactions* (the rat directs its snout towards the object stimulus and/or sniffs at the air in the same direction); *approach reactions* (the rat moves towards the object stimulus and contacts it with front paws and with the snout while actively sniffing); *consummatory attempts* (the rat licks and/or bites the object stimulus). Consummatory attempts do not result in actual consumption of Fonzies since the food is inside the box. The occurrence of each behavioral pattern was recorded during 40 min of box presentation subdivided in 5-min and 5-s block.

Behavior was scored by an observer unaware of the different treatment groups according to the following system (Bassareo and Di Chiara 1997): score 1, each orienting reaction for 5 s; score 2, same behavior for more than 5 s; score 3, each approach reaction for 5 s; score 4, same behavior for more than 5 s; score 5, each consummatory attempt for 5 s; score 6, same behavior for more than 5 s. The resulting scores were summed up for each 5-min period. Statistical analysis was carried out only on the first 5 min block of the behavioral reactions.

Histology

At the end of the experiment, probes were removed and the brains were kept in a 4% formaldehyde solution for at least 1 week and successively were cut by Vibratome in serial coronal slices oriented according to the atlas of Paxinos and Watson (1998). The location of the probes was reconstructed and referred to the atlas of Paxinos and Watson (1998; Fig. 1).

Statistics

Statistical analysis was carried out by Statistica for Windows. Depending on the experiments, data were analyzed by analysis of variance (ANOVA), with time as repeated measure (as in the case of the serial assays of dialysate DA) and training with Fonzies, chocolate, or morphine (as compared to standard food or saline) and presented with Fonzies or infused with chocolate solution

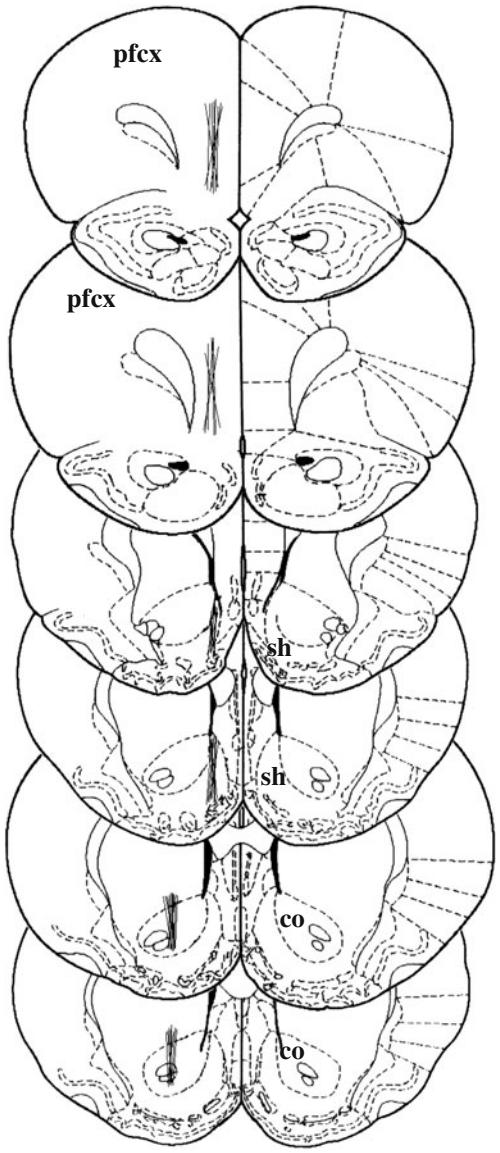

Fig. 1 Localization of dialysis probes within the PFCX, NAc shell, and core (according to Paxinos and Watson, 1998); *pfcx* prefrontal cortex, *sh* shell, *co* core

or challenge with morphine as between-subjects factors. Results from treatments showing significant overall changes were subjected to post hoc Tukey's test; $p<0.05$ was taken as statistically significant. Basal values were the means of three consecutive samples differing by no more than 10%.

Results

Basal values of DA (fmol, means±SEM) in 10-min samples were as follows: NAc shell, 52±5 (N=48); NAc core, 55±5 (N=34); PFCX, 14±2 (N = 25).

Food CS

Intrinsic Food CS: Fonzies-conditioned FB (N = 32)

Figure 2 shows the behavioral reactions (vertical bars) to FB and the changes in dialysate DA in response to FB and Fonzies presentation in the NAc shell, core, and PFCX in control and Fonzies-conditioned rats.

Behavioral reactions Two-way ANOVA of the behavioral reactions (vertical bars) to CS presentation showed an effect of areas ($F_{2,26}$=80.18; p=0.000001) of conditioning ($F_{1,26}$= 117,45; p=0.00001), and significant area×conditioning interaction ($F_{2,26}$=19.43; p=0.000007). Post hoc test showed a higher behavioral score in conditioned shell and PFCX groups as compared to control rats, and difference between shell- and core-conditioned group.

DA responses to the CS Three-way ANOVA of the changes in dialysate DA following CS presentation showed an effect of area ($F_{2,26}$=25.7; p=0.000001), conditioning ($F_{1,26}$=68.46; p=0.000001), time ($F_{3,104}$=28.24; p=0.000001), and an interaction of area×conditioning ($F_{2,26}$=18.2; p=0.000011), area×time ($F_{8,104}$=6.17; p=0.000002), conditioning×time ($F_{4,104}$=27.77; p=0.000001), and area×conditioning x time ($F_{8,104}$=13.26; p=0.000001). Post hoc Tukey's test showed a significant increase of DA in the NAc core and in the PFCX of conditioned rats, larger in conditioned rats with respect to control group and with respect to the conditioned shell group.

DA responses to the US Three-way ANOVA of the changes in dialysate DA following Fonzies presentation showed an effect of area ($F_{2,25}$=8.55; p=0.0015) and time ($F_{5,125}$= 49.16; p=0.00001) and an interaction of area×conditioning ($F_{2,25}$=17.52; p=0.000018), area×time ($F_{10,125}$=5.97; p= 0.000001), conditioning×time ($F_{5,125}$=7.38; p=0.000004) and area×conditioning×time ($F_{10,125}$=7.62; p=0.000001).

Post hoc test showed an increase of dialysate DA in the NAc core and in the PFCX of conditioned rats, in the PFCX of control group and lower dialysate DA in the NAc shell as compared to control group.

Extrinsic Food CS: chocolate-conditioned Fonzies box (N = 32)

Figure 3 shows the behavioral reactions (vertical bars) and the changes of NAc shell, core, and PFCX DA after FB presentation and intraoral chocolate in conditioned and control rats.

Behavioral reactions Two-way ANOVA of the behavioral reactions in response to FB presentation showed an effect of conditioning ($F_{1,27}$=67.51; p=0.00001), but no difference

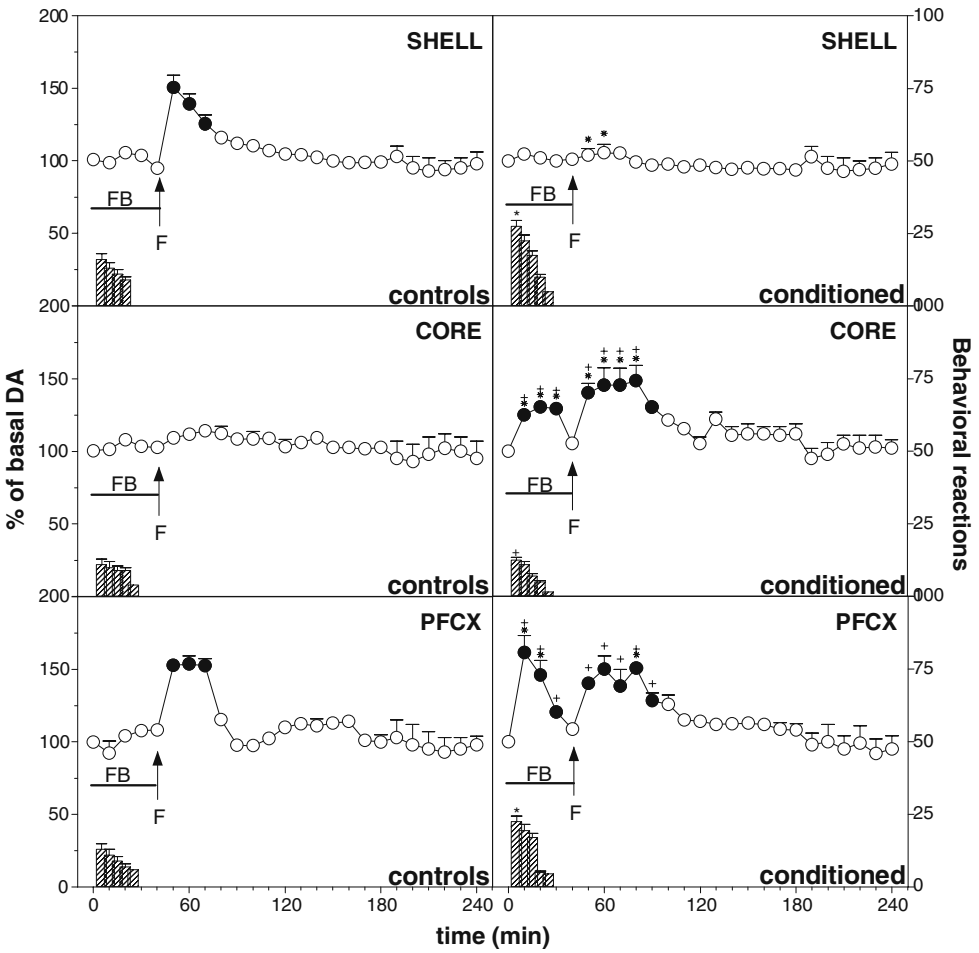

Fig. 2 Effect of FB and Fonzies meal on dialysate DA of the NAc shell, core, and PFCX of conditioned and control rats. Figure also shows incentive reactions after FB presentation during 40 min. Results are means±SEM of the results obtained in at least four rats. *Filled symbols* $p<0.05$ with respect to basal values; *asterisks* $p<0.05$ with respect to the correspondent control group; *positive signs* $p<0.05$ with respect to conditioned shell group

between areas ($F_{2,27}=1.29$; $p=0.29$) and no area×conditioning interaction ($F_{2,27}=2.51$; $p=0.1$). Post hoc test showed stronger behavioral reactions in conditioned compared to control rats.

DA responses to the CS Three-way ANOVA of the changes in dialysate DA following FB presentation showed an effect of time ($F_{4,108}=6.76$; $p=0.00068$) and an interaction of area×conditioning×time ($F_{8,108}=2.49$; $p=0.016$). Post hoc Tukey's test showed a significant increase of DA in the NAc core and in the PFCX of conditioned rats with a larger extent with respect to control groups.

DA responses to the US Figure 3 also shows the response of DA in the NAc shell, core, and PFCX after intraoral chocolate infusion.

Three-way ANOVA of the changes in response to intraoral chocolate showed an effect of area ($F_{2,26}=5.62$; $p=0.0094$) and time ($F_{8,208}=19.28$; $p=0.00001$) and an interaction of area×time ($F_{16,208}=3.52$; $p=0.000014$), conditioning×time ($F_{8,208}=3.36$; $p=0.0012$). Post hoc test showed a significant increase of DA in the NAc shell and in the PFCX of conditioned and control rats and in the NAc core of conditioned animals. Tukey's test also shows a higher increase of DA in the shell and core of conditioned compared with control rats.

Extrinsic Food CS: Fonzies-conditioned vanilla box (N = 8)

In order to investigate if the pattern of the effects of FB presentation on the responsiveness of DA were related to its intrinsic nature to the US, a box that contains within a cotton wad soaked with vanilla (VB) was used as CS.

Figure 4 shows the behavioral reactions (vertical bars) and the effect of VB and Fonzies on NAc shell DA in conditioned and control rats.

Behavioral reactions One-way ANOVA of the behavioral reactions to VB presentation showed an effect of conditioning ($F_{1,6}=30$; $p=0.0015$) and showed more pronounced behavioral reactions in conditioned compared to control rats.

DA responses to the CS Two-way ANOVA did not show any difference in the responsiveness of conditioned and control rats to VB ($F_{\text{conditioning}1,6}=1.42$; $p=0.28$; $F_{\text{time}4,24}=0.69$; $p=0.61$; $F_{\text{conditioning}\times\text{time}4,24}=0.93$; $p=0.45$).

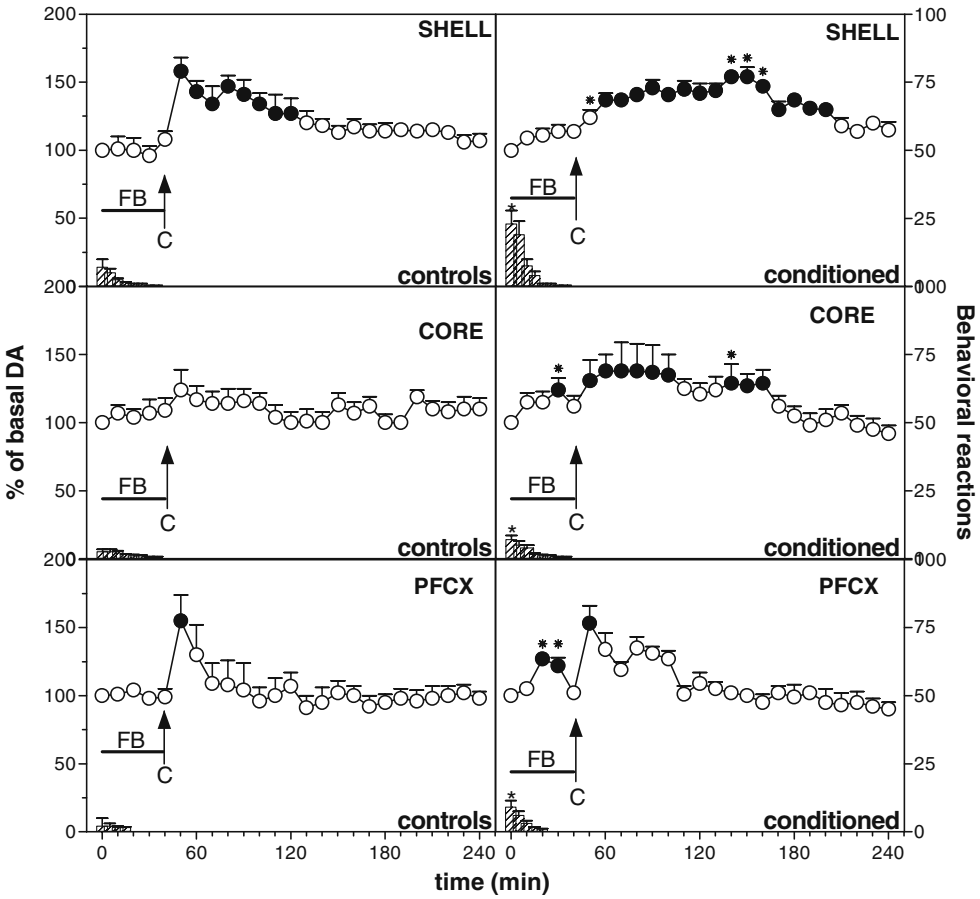

Fig. 3 Responsiveness of NAc shell, core, and PFCX DA of chocolate conditioned and control rats to FB and to intraoral chocolate. Incentive reactions after FB presentation during 40 min are also shown. Results are means± SEM of the results obtained in at least four rats. *Filled symbols* $p<0.05$ with respect to basal values; *asterisks* $p<0.05$ with respect to control group

DA responses to the US Two-way ANOVA showed an effect time ($F_{4,24}=70.66$; $p=0.00001$) and an interaction of conditioning×time ($F_{4,24}=16.95$; $p=0.00001$). Post hoc test showed a significant increase of DA in both groups, delayed in the conditioned rats.

Drug CS: morphine-conditioned vanilla box (N = 35)

Figure 5 shows the behavioral reactions (vertical bars) and the changes of NAc shell, core, and PFCX DA following VB exposure (CS) and morphine administration (US) in conditioned and control rats.

Behavioral reactions Two-way ANOVA of the behavioral reactions to VB presentation showed an effect of conditioning ($F_{1,29}=73.86$; $p=0.000001$), but did not show difference between areas ($F_{2,29}=3.32$; $p=0.06$) and no significant area×conditioning interaction ($F_{2,29}=2.74$; $p=0.08$). Post hoc test showed more pronounced behavioral reactions in conditioned with respect to control rats.

DA responses to the CS Three-way ANOVA of the changes in dialysate DA in response to VB presentation showed an effect of area ($F_{2,30}=11.91$; $p=0.00015$), conditioning ($F_{1,30}=58.99$; $p=0.000001$), time ($F_{4,120}=12.94$; $p=0.\ 0.000001$), and an interaction of area×conditioning ($F_{2,30}=4.21$; $p=0.024$), area×time ($F_{8,120}=3.84$; $p=0.0005$), and conditioning×time ($F_{4,120}=7.34$; $p=0.000025$). Post hoc Tukey's test showed a significant increase of DA in the NAc shell and in the PFCX of conditioned rats and also showed a larger increase in conditioned with respect to control rats implanted in these areas.

DA responses to the US Three-way ANOVA of the changes in dialysate DA in response to morphine administration showed an effect of area ($F_{2,28}=83.29$; $p=0.000001$), conditioning ($F_{1,28}=9.72$; p=0.0042), and time ($F_{12,336}=24.87$; p=0.00001) and an interaction area×conditioning×time ($F_{24,336}=3.32$; $p=0.000001$). Post hoc test showed a significant increase of DA in all the three areas monitored of conditioned groups and in the shell of control group. Tukey test also shows that the increase of DA in the shell and in the PFCX of conditioned rats was stronger compared to that obtained in control groups.

Comparison between DA responses to VB–Fonzies conditioned and to VB–morphine conditioned Two-way ANOVA of the changes in dialysate DA following VB presentation in VB–Fonzies and VB–drug groups showed an effect of

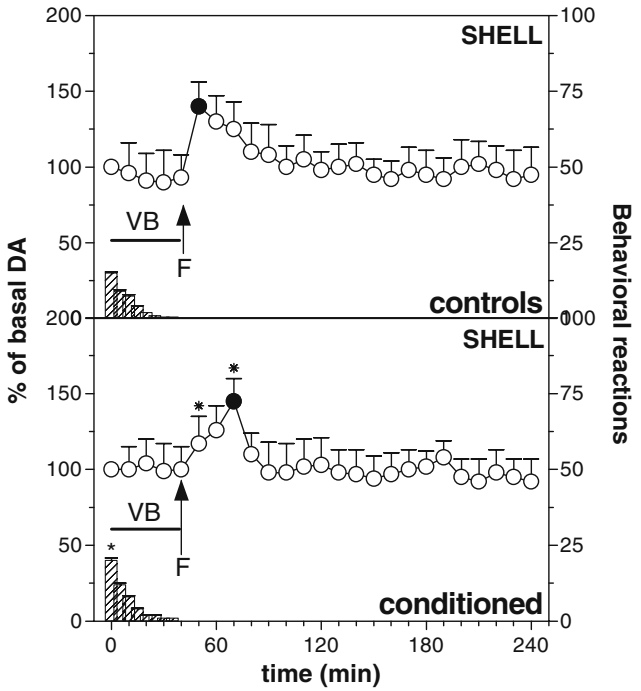

Fig. 4 Effect of VB and Fonzies feeding on dialysate DA of the NAc shell of conditioned and control rats. Figure also shows incentive reactions after VB presentation during 40 min. Results are means± SEM of the results obtained in at least four rats. *Filled symbols* $p<0.05$ with respect to basal values; *asterisks* $p<0.05$ with respect to the correspondent control group

kind of US associated with the CS ($F_{1,9}=30.52$; $p=0.0004$), time ($F_{4,36}=6.13$; $p=0.0007$) and an interaction of kind of US×time ($F_{4,36}=6.11$; $p=0.0007$). Post hoc Tukey's test showed a significant increase of DA shell only in the VB–drug group.

Discussion

In the present conditioning procedure, the CS does not overlap with the US, a feature of trace conditioning (Woodruff-Pak and Disterhoft 2008). Conditioning was indicated by the fact that in all groups of animals, except for the FB+F group implanted in the NAc core, stimuli elicited higher score incentive responses in conditioned as compared to control rats. In conditioned subjects, these behavioral responses were associated to increase in dialysate DA in the PFCX. This observation supports the notion that stimulation of PFCX DA is an expression of the impact of motivationally significant stimuli independently of their conditioned or unconditioned nature and affective valence (appetitive or aversive; Bassareo and Di Chiara 1997). Therefore, the ability of CSs, both food- and drug-conditioned, to activate PFCX DA can be regarded as a further indication of their efficacy as conditioned stimuli.

The circumstance that conditioning did not increase incentive reactions in rats implanted in the NAc core could be due to the fact that the insertion of the microdialysis probe may have damaged this area. This observation suggests that the NAc core is important for the expression of the conditioned incentive responses, consitent with the conclusions of Parkinson et al. (1999).

In the present study, direct comparison between the effects of food– and drug–CSs shows that complex, mainly olfactory, CSs differentially affect NAc shell and core DA transmission depending on the fact that they have been food-conditioned to food or to drug (morphine) USs. Thus, food–CSs increase extracellular DA in the NAc core but not in the shell while drug–CSs increase DA in the NAc shell but not in the core. PFCX DA transmission, instead, was similarly activated by drug and food CSs (Bassareo et al. 2007). We cannot exclude that some activation of DA transmission can take place in the shell in response to food–CSs. However, if this is the case, the change is below the sensitivity of the detection procedure and lowers than that in the core.

In the present study, we have utilized as drug–CS a plastic box carrying, in place of Fonzies (FB), as in our previous study (Bassareo et al. 2007), a wad soaked with vanilla solution (VB). Like FB (Bassareo et al. 2007), VB presentation activated DA transmission in the NAc shell, and in the PFCX but not in the NAc core. This observation indicates that the effect of food- and drug-conditioned stimuli on DA transmission is related to the nature of the US rather than of the CS.

The present studies and the relative results, while consistent with previous observations from our laboratory, show a number of differences with studies from other laboratories.

Ito et al. (2000) showed that a discrete (light) CS previously paired to i.v. cocaine self-administration, increased dialysate DA in the NAc core when presented in a response non-contingent manner. No DA response was obtained in the NAc shell. These observations stand in contrast with the present ones. However , these differences can be explained by the different nature of conditioning and different kind of the stimulus utilized as CS, instrumental and discrete in the case of Ito et al. (2000), Pavlovian and contextual/olfactory, in the present study. We have recently completed a microdialysis study comparing changes in extracellular DA in the NAc shell and core of rats nose-poking for sucrose pellets and we found that non-contingent presentation of an instrumental discrete (light) CS resulted in a sharp DA response in the NAc shell but not in the NAc core (Bassareo et al. 2009). Parallel studies with the same instrumental paradigm and CS but utilizing fast-scan cyclic voltammetry have shown a higher and longer-lasting release of DA in the shell as compared to the core (Cacciapaglia et al. 2008). Similar considerations, as to the instrumental feature of the conditioning and the discrete

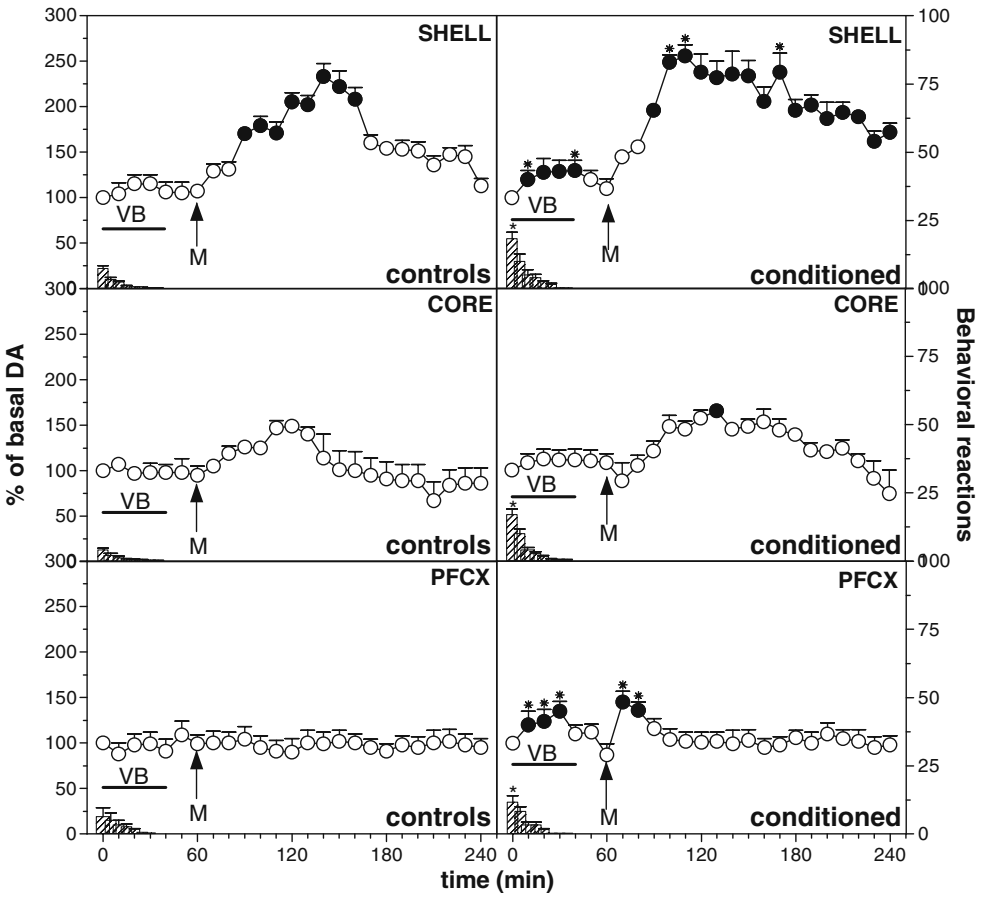

Fig. 5 Impact of VB and morphine challenge on dialysate DA of the NAc shell, core, and PFCX of conditioned and control rats. Figure also shows incentive reactions after VB presentation during 40 min. Results are means±SEM of the results obtained in at least four rats. *Filled symbols* $p<0.05$ with respect to basal values; *asterisks* $p<0.05$ with respect to the correspondent control group

nature of the CS, apply to the voltammetric studies of Phillips et al. (2003) and Roitman et al. (2004) who observed a phasic increase of DA in the NAc core, peaking 1–3 s after cue presentation and returning to basal after 3–6 s in rats bar pressing for cocaine and, respectively, for food. Due to the differential nature and the subsecond time scale, voltammetric measurements are not easily compared with microdialysis that provides an absolute estimate of DA on a minute time scale. However, it should be pointed out that, taking into account the above differences, under similar experimental conditions, the observations made with voltammetry (Cacciapaglia et al. 2008; Aragona et al. 2009) basically agree with those obtained with microdialysis (Di Chiara and Bassareo 2007; Bassareo et al. 2009).

More relevant for the present observations might be the study of Cheng et al. (2003) on the effect of a tone CS paired to non-contingent food pellet presentation on dialysate DA in the NAc shell and core. Rats showed strong nose poking into the magazine food and locomotion in response to the CS and, in contrast to the present study, a similar increase of DA in the NAc shell and core. However, there are various differences between the study by Cheng et al. (2003) and the present one that might account for their failure to observe differences in the responsiveness of NAc shell and core DA to the CS. First, rats were not fed ad libitum, as in our case, but were maintained with 14 g per day of standard food, that kept their body weight at 90% of the fed ad libitum level, starting 3 days before the beginning of the behavioral sessions and for the following 4 days, i.e., for 1 week before the microdialysis experiment; food was given only after completion of daily training, i.e., 7 h after starting of the light-off period. Second, the CS was a discrete, unimodal (auditory) stimulus rather than a multimodal contextual/olfactory stimulus. Third, rats were trained for 3 days on three daily acquisition sessions each made up of six CS–US trials. Therefore, microdialysis was performed after as many as 54 CS–US pairings. In the present study, as little as three CS–US pairings were sufficient to obtain conditioning. It is clear that the study of Cheng et al. (2003) utilized classical conditioning in a Skinner box, a procedure that has the advantage of strictly controlled conditions but involves a certain degree of stress related to the combination of food restriction and scheduled (10-s interval) food presentation and a much longer training procedure. On the other hand, we have shown that even mild food restriction abolishes the habituation of DA response to food, a property specific to shell DA (Bassareo and Di Chiara 1997). Given the postulated role of NAc shell DA in incentive learning, food restriction can be

expected to affect the acquisition of CS, in addition to increase the incentive value of food.

Collectively, these observations suggest that the differential response of DA transmission to CSs in the shell versus core depends on specific experimental conditions such as nature of the US (drug versus food) and of the CS (discrete versus contextual/olfactory), deprivation state, and type of conditioning (instrumental versus pavlovian).

CSs, in addition to directly affect DA transmission, do affect the responsiveness of DA transmission to the US. In the present study, we have confirmed our previous observations (Bassareo and Di Chiara 1997, 1999b; Bassareo et al. 2007) by utilizing for acquisition and scoring the same schedule and method applied to drug-conditioning (Bassareo et al. 2007). In addition, we have extended the observation of a strengthening effect of CS pre-exposure on the response of NAc shell and core DA to the drug US by utilizing a CS (VB) different from the one utilized in our previous study (Bassareo et al. 2007).

Under these conditions, exposure to FB conditioned to Fonzies feeding inhibits the response of NAc shell to the US. No such inhibition was observed in the PFCX. In the NAc core, pre-exposure to the intrinsic CS actually increased the DA response (Bassareo and Di Chiara 1999a). On the other hand, pre-exposure to the drug–CS potentiates the response to the drug US in the NAc shell (Bassareo et al. 2007).

How do we explain the clear-cut difference between food– and drug–CSs vis-a-vis their influence on the response of NAc shell DA to the US? Here, a basic, preliminary issue is that of the differences and similarities of the sensory properties of the CS and of the US in the case of food as compared to drug conditioning.

Indeed, while conditioned stimuli (FB) have quite different sensory properties from those of the drug US, this is not the case when Fonzies is the US: in this case, the CS has some salient sensory properties (mainly olfactory) in common with the US. Thus, while in the case of drug-conditioned FB and VB, the CS is totally extrinsic to the US, in the case of Fonzies-conditioned FB, the CS is intrinsic to the US. In this last case, exposure to the intrinsic CS might mimic the US in eliciting a reduction of the response of NAc shell DA to the US similar to that elicited by pre-exposure to the US itself (Bassareo and Di Chiara 1997).

If indeed the inhibitory effect of the food CS on the response to the US is due to generalization to the CS of the properties of the US (habituation), one would expect that no such inhibition of NAc shell DA response would take place if the CS is provided with stimulus properties different from those of the US. In view of this, we tested the habituation hypothesis in two ways: by testing the effect of a different CS (VB) and keeping the same US (Fonzies feeding) or changing the US (intraoral sweet chocolate) and keeping the same CS (FB).

Consistent with the above hypothesis, in both cases pre-exposure to the CS did not inhibit the stimulatory effect of food on NAc shell DA. Conversely, exposure to the extrinsic CS, not only did not inhibit but actually prolonged the stimulation of the NAc shell by the US and resulted in activation of DA transmission in the core.

We therefore conclude that the inhibition of the NAc shell DA response by an intrinsic food–CS is due to generalization to the CS of the phenomenon of habituation of NAc shell DA responsiveness to repeated exposure to the same food US (Bassareo and Di Chiara 1997).

The effects of feeding on DA transmission are mainly pre-ingestive, being related to their taste properties, as indicated by their short latency and time course. This is also consistent with the observations of Hajnal et al. (2004) who reported that drinking of sucrose solutions increased DA in the NAc in rats with gastric fistulas that prevent post-ingestive effects but the same solutions fail to do so when infused directly into the stomach. Nonetheless, food exerts rewarding effects also as a result of its post-ingestive properties (Sclafani and Ackroff 1994). Although these properties are not accompanied by activation of DA transmission (see above), we cannot exclude that food reward related to post-ingestive effects are able to condition DA transmission in areas, such as the NAc core, that are unresponsive to the US itself.

The failure of food–CSs to activate DA in the NAc shell, although puzzling, is nonetheless solid and therefore might provide clues for understanding the role of DA in the properties of CSs. A basic property of reward CSs is the ability to elicit incentive responses. Ventral striatal DA has been suggested to be involved in the expression of incentive–motivational properties of stimuli (Blackburn et al. 1992; Robinson and Berridge 1993). The basic form of these incentive responses consists of simple approach, the main component of the incentive response to the CSs observed in the present study. Our observations, however, suggest that, under the present conditions, NAc shell DA is not involved in the expression of simple incentive responses by food CSs. Such role, instead, would apply to drug–CSs, that are able to activate DA transmission in the NAc shell. Therefore, it is possible that, under the present experimental conditions, NAc shell DA is differentially involved in the incentive reactions induced by food– as compared to drug–CSs.

As to drug-conditioned stimuli, the present results extend to a different CS, vanilla box, the observations previously obtained with a Fonzies Box as a CS (Bassareo et al. 2007). Thus, the CS increased NAc shell and PFCX DA but not NAc core DA and strengthened the response of DA to morphine in all the three areas.

From the present and previous studies, it appears that the most clear-cut differences between the effects of food– versus drug–CSs on DA transmission are observed in their effects on NAc shell and core DA. In these areas, drug– and food–CSs exert reciprocal effects: while drug–CSs increase DA in the shell but not in the core, food–CSs increase DA in the core but not in the shell.

The reason for the above differences is unclear and open to speculation. A first possibility is that the different shell/core specificity of the DA stimulant properties of food– versus drug–CSs is the result of different stimulus processing. Thus, contextual stimuli are processed via the hippocampus/shell pathway while discrete stimuli via the basolateral amygdala/core pathway (Fuchs et al. 2009; Ramirez et al. 2009). Moreover, drug and food USs differ in the timing, duration, and extent of their stimulant properties on DA transmission; these differences are particularly pronounced in the NAc shell, where drugs activate DA transmission to a larger extent than food and for a much longer duration. These differences might affect the acquisition of conditioned DA stimulant properties of the CSs, consistently with the postulated role of NAc shell DA in associative learning (Di Chiara 2002).

Another difference between morphine and food USs that might be relevant for the differential impact of their respective CSs on NAc shell and core DA is in the topography of their DA stimulant effects. Thus, morphine increases DA preferentially and, depending on the dose, selectively in the NAc shell versus core and does not affect PFCX DA (Bassareo et al. 2007). Palatable food also tends to produce a larger effect in the shell compared to the core but this difference is not as clear-cut as in the case of morphine, that in turn fails to increase DA in the PFCX (Bassareo et al. 1997; Gambarana et al. 2003; Liang et al. 2006; Danielli et al. 2009; Pontieri et al. 1995; Cadoni and Di Chiara 1999).

The differential effect of drug– versus food–CSs on NAc shell DA might be also the result of the dysadaptive properties of drug-induced as compared to food-induced stimulation of DA transmission in the NAc shell, namely, of the resistance to habituation of NAc shell DA transmission upon repeated exposure to the US (see Di Chiara and Bassareo 2007 for discussion). This property of drugs of abuse is expected to facilitate the acquisition of drug–CSs by reducing the number of CS–US associations needed for successful conditioning, to increase the incentive properties of the CS, to retard extinction, and to increase reinstatement upon re-exposure to the US (priming). Given the importance attributed to drug CSs in the mechanism of drug addiction, these aspects might be of prime importance for the mechanism of the initial stages of drug addiction (Di Chiara 1998).

The present observation that drug CSs, in contrast to food CSs, activate DA transmission in the NAc shell might be particularly relevant for the mechanism of drug addiction and might be incorporated into an incentive learning theory of this condition. We have already proposed that drugs of abuse, by releasing DA in the NAc shell in a non-habituating fashion, abnormally facilitate learning of drug-conditioned incentives that are instrumental to initiate and maintain drug seeking (Di Chiara et al. 2004; Di Chiara 2002). Now, the present observations suggest that drug-conditioned incentives, by releasing DA in the NAc shell, facilitate the further acquisition of secondary incentives, thus inducing the formation of a chain of CSs that ultimately contribute to maintain drug consumption. This theory might be extended to explain disturbances of food seeking, such as compulsive overeating, as the result of the existence, in certain individuals, of an abnormality in the DA-stimulant properties of food CSs, namely, the property to release DA in the NAc shell (Di Chiara 2005).

References

Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM (2008) Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. J Neurosci 28(35):8821–8831

Aragona BJ, Day JJ, Roitman MF, Cleaveland NA, Wightman RM, Carelli RM (2009) Regional specificity in the real-time development of phasic dopamine transmission patterns during acquisition of a cue–cocaine association in rats. E J Neurosci 30:1889–1899

Avena MN, Rada P, Hoebel BG (2008) Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci Biobehav Rev 32(1):20–39

Bassareo V, Di Chiara G (1997) Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci 17(2):851–861

Bassareo V, Di Chiara G (1999a) Differential responsiveness of DA transmission to food stimuli in nucleus accumbens shell/core compartments. Neurosci 89(3):637–641

Bassareo V, Di Chiara G (1999b) Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. Eur J Neurosci 11:4389–4397

Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G (2003) Differential adaptive properties of accumbens shell dopamine response to ethanol as a drug and as a motivational stimulus. Eur J Neurosci 17(7):1465–1472

Bassareo V, De Luca MA, Di Chiara G (2007) Differential impact of pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. Psychopharmacology 191:689–703

Bassareo V, Musio P, Lecca D, Di Chiara G (2009) Mesolimbic dopamine responsiveness to food conditioned stimuli after instrumental conditioning paradigm. Behav Pharmacol 20(special issue):S37, Abstracts of the 13th Biennal EBPS meeting

Blackburn JR, Pfaus JG, Phillips AG (1992) Dopamine functions in appetitive and defensive behaviours. Prog Neurobiol 39(3):247–279

Cacciapaglia F, Owesson-White CA, Wheeler RA, Wightman RM, Carelli RM (2008) Nucleus accumbens cell firing and rapid dopamine release during food-seeking behavior in rats. Society for Neuroscience Abstract, Washington DC

Cadoni C, Di Chiara G (1999) Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. Neurosci 90(2):447–455

Cheng JJ, de Bruin JPC, Feenstra MGP (2003) Dopamine efflux in nucleus accumbens shell and core in response to appetitive classical conditioning. E J Neurosci 18:1306–1314

Ciccocioppo R, Martin-Fardon R, Weiss F (2002) Effect of selective blockade of μ_1 and δ opioid receptors on reinstatement of alcohol-seeking behavior by drug-associated stimuli in rats. Neuropsychopharmacology 27(3):391–399

Danielli B, Scheggi S, Grappi S, Marchese G, De Montis MG, Tagliamonte A, Gambarana C (2009) Modifications in DARPP-32 phosphorylation pattern after repeated palatable food consumption undergo rapid habituation in the nucleus accumbens shell of non food-deprived rats. J Neurochem 112 (2):531–541

Di Chiara G (1998) A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. J Psychopharmacol 12(1):54–67

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137:75–114

Di Chiara G (2005) Dopamine in disturbance of food and drug motivated behavior: a case of homology? Physiol Behav 86:9–10

Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol 7:69–76

Di Chiara G, Tanda G, Frau R, Carboni E (1993) On the preferential release of dopamine in the nucleus accumbens by amphetamine: further evidence obtained by vertically implanted concentric dialysis probes. Psychopharmacol 112:98–402

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47(Suppl 1):227–241

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala–ventral striatal subsystems. Ann n Y Acad Sci 877:412–438

Fenu S, Spina L, Rivas E, Di Chiara G (2006) Morphine-conditioned single-trial place preference: role of nucleus accumbens shell dopamine receptors in acquisition, but not expression. Psychopharmacol Berl 187:143–153

Fuchs RA, Bell GH, Ramirez DR, Eaddy JL, Su ZI (2009) Basolateral amygdala involvement in memory reconsolidation processes that facilitate drugcontext-induced cocaine seeking. Eur J Neurosi 30:889–900

Gambarana C, Masi M, Leggio B, Grappi S, Nanni G, Scheggi S, De Montis MG, Tagliamonte A (2003) Acquisition of a palatable food-sustained appetitive behavior in satiated rats is dependent on the dopaminergic response to this food in limbic areas. Neuroscience 121:179–187

Hajnal A, Smith GP, Norgren R (2004) Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol 286:R31–R37

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 20:7489–7495

Liang NC, Hajnal A, Norgren R (2006) Sham feeding corn oil increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol 291:R1236–R1239

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of nucleus accumbens core and shell in appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 19:2401–2411

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates IV ed Academic Press New York

Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. Nature 422(6932):614–618

Pontieri FE, Tanda G, Di Chiara G (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci USA 92:12304–12308

Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255–257

Rada P, Avena NM, Hoebel BG (2005) Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 134:737–744

Ramirez DR, Bell GH, Lasseter HC, Xie X, Traina SA, Fuchs RA (2009) Dorsal hippocampal regulation of memory reconsolidationprocesses that facilitate drug context-induced cocaine-seeking behavior in rats. Eur J Neurosci 30:901–912

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive–sensitization theory of addiction. Brain Res Rev 18:247–291

Roitman MR, Phillips PEM, Stuber G, Wightman RM, Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. J Neurosci 24(6):1265–1271

Sclafani A, Ackroff K (1994) Glucose- and fructose-conditioned flavor preferences in rats: taste versus post-ingestive conditioning. Physiol Behav 56:399–405

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacol Berl 168(1-2):3–20

Spina L, Fenu S, Rivas E, Di Chiara G (2006) Nicotine-conditioned single-trial place preference: selective role of nucleus accumbens shell dopamine D1 receptors in acquisition. Psychopharmacol Berl 84:447–455

Stewart J, de Wit H, Eikelboom R (1984) Role of unconditioned and conditioned drug affects in the self-administration of opiates and stimulants. Psychol Rev 91:251–268

Tanda G, Bassareo V, Di Chiara G (1996) Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. Psychopharmacol 123:127–130

Tanda G, Pontieri FE, Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048–2050

Volkow ND, Wang GJ, Ma Y, Fowler JS, Zhu W, Maynard L, Telang F, Vaska P, Ding YS, Wong C, Swanson JM (2003) Expectation enhances the regional brain metabolic and reinforcing effects of stimulants in cocaine abusers. J Neurosci 23(36):11461–11468

Woodruff-Pak DS, Disterhoft JF (2008) Where is the trace in trace conditioning? Trends Neurosci 31(2):105–112