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Dopamine responsiveness in Nucleus Accumbens Shell and Core and Prefrontal Cortex during operant behavior for sucrose

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Presented by: Flavia Cucca

PhD Co-ordinator: Prof Gaetano Di Chiara

Supervisor: Prof Gaetano Di Chiara

Tutor: Dr Valentina Bassareo

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1. Introduction

1.1 Dopamine and reward

For better and for worse dopamine (DA) has been linked to reward.

In the late 70's, Wise (1980) proposed DA as the substrate of all rewards, either conventional (food, water, sex), pharmacological (drugs of abuse) and physical (intracranial self-stimulation). This anhedonia hypothesis was soon contrasted by hypotheses assigning to DA an incentive-motivational (Berridge and Robinson, 1998) and / or an activational role (Salamone, 1992) but negating a hedonic role. However, an incentive-motivational role of DA is not incompatible a priori with a hedonic role. Thus, Wise himself revised his original anhedonia hypothesis and extended the role of DA to that of a substrate of the hedonic properties of incentive stimuli (Wise, 1980). According to this proposal, DA mediates not only the pleasurable properties of primary rewards but also of stimuli (incentives) that derive their motivational properties from having been conditioned to rewards (Wise, 1980). Although this proposal has not given much attention in the past, the idea of DA as a mediator of the pleasurable properties of conditioned incentive-motivational stimuli has been revived in the hypothesis of a role of nucleus accumbens (NAc) shell DA in hedonia associated to incentive arousal, the state induced by incentive stimuli (Di Chiara, 2002). Incentive arousal can be regarded as a state of heightened mood (high, euphoria) by which incentives energize behavior, thus promoting the search, pursuit and approach of rewards. Theories negating a role of DA in hedonia fail to account for the pleasurable, hedonic and ultimately reinforcing properties of psychostimulants like amphetamine and cocaine. In fact, these drugs provide a unique model of the incentive arousal state and of its hedonic quality. If incentive arousal, including its hedonic quality, is equated to euphoria, evidence for the above proposal is provided in humans by PET studies showing that the intensity of euphoria induced by amphetamine is related to reduction of C11-raclopride binding potential in the ventral striatum (Drevets et al, 2001). The hypothesis of a role of DA in state hedonia is not excluded by and can coexist with incentive-motivational hypotheses excluding a role of DA in hedonia. Indeed, these hypotheses refer to a kind of pleasurable stimuli quite distinct from those that induce incentive arousal and state hedonia and that belong to different aspects of motivated behavior. Thus, state hedonia takes place during the preparatory/appetitive phase of motivated behavior and is elicited by distal stimuli, i.e., stimuli that do not involve contact with the subject (olfactory, auditory, visual, ultrasonic stimuli). Appetitive hedonia and incentive hedonia are synonymous of state hedonia. On the other hand, sensory hedonia takes place during the consummatory phase of motivated behavior and is elicited by proximal stimuli, i.e., stimuli that involve direct contact with the subject (gustatory, tactile, proprioceptive). Consummatory hedonia is

synonymous of sensory hedonia. The above proposal, by envisioning a strict relationship between DA and incentive-motivation, might seem just analogous to incentive-motivational theories of the role of DA in behavior. This however would be erroneous. Thus, while attributing to DA a role in providing incentive value for conditioned stimuli, those theories do exclude a role of DA in stimulus valence. Depending on its sign, positive or negative, stimulus valence determines the direction of the incentive response. Thus, while positive incentives determine approach, negative incentives elicit avoidance. In our proposal, DA, released in the ventral striatum/ NAc shell by incentives, adds a positive valence to the motivational properties of stimuli, thus promoting approach towards the stimulus. DA might also be involved in the incentive value of stimuli, although this is not the original aspect of our hypothesis. In this case, both properties of incentive stimuli, hedonic valence and incentive value, might be inextricably linked by their dependence on DA. Indeed, a tight association between hedonic and motivational aspects of incentive stimuli corresponds to early formulations of incentive-motivational theories (Bindra, 1974). A dissociation between these aspects has been advanced by later theories (Berridge and Robinson, 1998). However, in these theories, hedonic properties were envisioned as belonging to consummatory stimuli and not to incentive ones.

The assignment of DA-dependent hedonic properties to incentive stimuli and its proposed relationship with mood state allows translation into clinically relevant issues. Thus, according to this framework, anhedonia refers to a mood state ultimately related to a reduction of the tone of DA transmission in the shell of the NAc. This makes the term anhedonia, as derived from animal studies, homologous to the same term utilized in the context of mood disorders. This meaning, in turn, is quite different from that attributed to it by Wise (1980), both in the original and in the revised anhedonia hypothesis, where no distinction between incentive and consummatory hedonia had been made.

1.2 Dopamine transmission and behavior

DA acts via G-protein-coupled receptors in a typical neuromodulatory fashion (Greengard, 2001). DA release sites are placed immediately outside the synaptic cleft (Sesack et al, 2003). Once released, DA diffuses in the extracellular fluid, from which it is slowly cleared as a result of reuptake and metabolism (Venton et al, 2004). DA does not directly affect the conductance of receptive membranes but modifies their response to afferent input (O'Donnel, 2003). These three aspects (extrasynaptic release, G-protein-coupled receptor signal transduction and a modulatory mechanism) contribute to a basic feature of DA transmission; that is, the long delay occurring between stimulus-bound activity (burst firing) and functional changes in the receptive elements. It

has been estimated that, following electrical stimulation of DA neurons, a change in activity is recorded in striatal neurons after a delay of approximately 300 ms (Gonon, 1997). Although burst firing of DA neurons occurs in response to motivationally relevant stimuli (Schultz, 2002), it is unlikely that these phasic DA signals influence, to any significant extent, the behavioral response (mediated by fast transmitting pathways) to the same stimulus that triggered them. Thus, a more realistic view of the role of DA in responding involves DA as a delayed amplifier of responding, affecting the behavioral impact of stimuli that follow the one that triggered its release. Recent fast-scan cyclic voltammetry studies support this contention. Thus, in rats responding for sucrose (Roitman et al, 2004) or intravenous cocaine (Phillips et al, 2003), the largest DA transient recorded in the nucleus accumbens (NAc) core peaked either at the start (sucrose) of the response or 1–2s thereafter (cocaine). Therefore, rather than being 'in series' between stimuli and responses, DA should be envisioned in parallel with stimuli, modulating their ability to elicit a response (Di Chiara, 2002).

1.3 In vivo monitoring of dopamine transmission

DA function can be monitored by extracellular recording of the firing activity of DA neurons (Schultz, 2002) and by estimating the extracellular concentrations of DA by microdialysis (Volkow et al, 2003; Chang and Haning, 2006), voltammetry (Robinson et al, 2003) and brain imaging (i.e. positron emission tomography [PET]) (Volkow et al, 2003; Chang and Haning, 2006). Each of these methods has different time frames: milliseconds for extracellular recordings, seconds for voltammetry, and minutes for microdialysis and PET. These different methods do not necessarily estimate the same aspect of the function of DA. It has been proposed that DA operates in different modalities depending upon the time-scale of its action (Grace, 2000; Lavin et al, 2005). Thus, a phasic modality, operating in a time-frame of hundreds of milliseconds and related to release of DA by a burst of spikes onto low affinity DA receptors, has been distinguished from a tonic modality, operating in a circadian time-frame and related to the basal steady-state concentration of DA in the extracellular compartment arising from the dilution and diffusion of released DA. The phasic modality corresponds to DA transients estimated by voltammetry, the tonic modality to basal DA concentrations estimated by microdialysis (Grace, 2000). This dichotomous categorization, however, is insufficient to describe the changes in the minute time-frame observed by microdialysis and PET in response to reward-related stimuli. Therefore, a more comprehensive model envisions the existence of multiple time-related modalities of DA transmission that depend upon the number of bursts fired by specific pools of DA neurons (Sesack et al, 2003).

Microdialysis studies in the rat have shown that appetitive taste stimuli release DA in the NAc shell and core, as well as in the prefrontal cortex (PFCX) (Bassareo and Di Chiara, 1997; Hajnal et al, 2004). NAc shell DA responsiveness shows some differences to that of the NAc core and PFCX, as it is dependent upon the hedonic valence (appetitive or aversive) (Bassareo et al, 2002) and relative novelty of taste stimuli (Bassareo and Di Chiara, 1997; Bassareo et al, 2002; Bassareo and Di Chiara, 1999a). Thus, NAc shell DA release is stimulated by unfamiliar appetitive tastes, but is unaffected or even decreased by aversive tastes (Bassareo et al, 2002). NAc shell DA responsiveness habituates after a single exposure to palatable food in a taste-specific manner (Bassareo and Di Chiara, 1997; Bassareo et al, 2002; Bassareo and Di Chiara 1999b). By contrast, taste stimuli release DA in the NAc core and in the PFCX independently of their positive or negative hedonic valence, and do not show single-trial habituation (Bassareo and Di Chiara, 1997; Bassareo et al, 2002). Mild food deprivation is sufficient to impair habituation of NAc shell DA responsiveness to palatable food (Bassareo and Di Chiara, 1999b); this could account for the failure of DA neurons to undergo habituation in food- restricted monkeys (Schultz, 2002). Habituation of the DA response to intraoral sweet chocolate is not associated with reduction in hedonic taste reactions (Bassareo et al, 2002). This indicates that habituation is unrelated to satiety-induced hedonic devaluation and, in turn, that hedonic taste reactions are independent of NAc shell DA. Accordingly, DA release in the NAc shell is not the cause, but the consequence, of food reward. The adaptive properties of the responsiveness of NAc shell DA to taste stimuli (one-trial habituation) are consistent with a role in associative learning (Bassareo and Di Chiara, 1997). Consistent with this suggestion, intra-NAc shell infusion of D1 receptor antagonists impairs acquisition of conditioned taste aversion, whereas systemic amphetamine facilitates this process by an action in the NAc shell (Fenu et al, 2001; Fenu and Di Chiara, 2003). Therefore, release of DA in the NAc shell following food intake might serve to associate the taste properties of food to its post-ingestive consequences (Fenu et al, 2001).

1.4 Nucleus Accumbens

The last quarter of the past century has seen a renewed interest in brain areas that belong to what we like to refer to as ‘the basement of the brain’. These ventrally and medially located areas include the ventral striatum and in particular the shell of the nucleus accumbens septi (NAc), as well as a number of nuclei that are part of the archistriatum (Alheid and Heimer, 1988; Heimer et al, 1991; Heimer and Wilson, 1975). Because of their strong homologies and reciprocal connections, these areas have been grouped into a complex, the extended amygdala, that includes the central amygdala, bed nucleus of stria terminalis, sublenticular substantia innnominata and intercalated

grey masses (Alheid and Heimer, 1988; Heimer et al, 1991).

This nuclear complex corresponds to the oldest and most mysterious part of the forebrain, the one that, in contrast to the upper and more recent domains, has not undergone any major change throughout evolution. Also like any basement, the NAc shell/extended amygdala complex is full of those good-old-things that are kept, 'just in case' and turn out to be essential in exceptional, unpredictable circumstances. In the basement of the brain this dismissed merchandise corresponds to behavioral functions and response sets essential for the survival of the self and of the species. These functions can be grouped collectively under the heading of 'motivation', which refers to the ability, unique to living organisms, to respond to stimuli in relation to their individual needs and with the ultimate goal of the survival of their own species.

The NAc is a heterogeneous structure. Its ventro-medial portion, the shell has been regarded as a transition area to the Extended Amygdala (Heimer et al.,1991; Jongen-Relo et al.,1994; Heimer et al.,1997), while the dorso-lateral portion, the core, is considered as an extension of the Caudate-Putamen and to subserve a motor function (Stolerman, 1992; Alheid e Heimer,1988; Zahm e Brog, 1992; Groenewegen and Russchen, 1989; Voorn et al., 1989; Heimer et al., 1991). The NAc has been suggested to provide an interface between limbic and motor functions (Mogenson et al., 1980; Mogenson & Yang, 1991) and to be involved in several aspects of behavior such as motivation, reward, water and food intake, sexual behavior and to play an important role in drug dependence (Wise, 1987; Alexander & Crutcher, 1990; Alheid et al., 1990, Smith and Bolam, 1990, Di Chiara, 2002; Di Chiara et al., 2004; Di Chiara & Bassareo, 2007; Anselme, 2009). The shell of the NAc has been attributed a role in the acquisition and expression of incentive motivation while the core compartment is involved mainly in the motor expression of motivated behaviour (Zahm & Brog, 1992; Bassareo & Di Chiara, 1999; Berridge and Robinson, 1998, Brauer et al., 2000).

1.5 Medial Prefrontal Cortex

The prefrontal cortex (PFCX) can be distinguished into three main regions: the dorsolateral (Brodman areas 9 and 46), the inferior ventral (areas 11, 12, 13 and 14 – also known as orbitofrontal cortex) and the medial portion (mPFCX) , distinguished into a dorsal, prelimbic, and a ventral, infralimbic, area.

Each subarea of the mPFCX has distinct afferent and efferent connections. This anatomic heterogeneity reflects the different function such as planning of voluntary action, arousal and attention, temporal sequencing of actions, planning of forthcoming behaviour based on previously acquired information, response selection and response inhibition (Pinel, 2000; Tzschentke, 2001;

Curtis and D'Esposito, 2003; Kolb and Whishaw, 2003; Ramnani and Owen, 2004; Surmeier, 2007).

Basal extracellular and tissue levels of DA in the mPFCX are low, due to the fact that in this area the DAergic innervation is less dense in respect to other DAergic areas like NAc.

The mesocortical DAergic system is particularly responsive to stress, both acute and chronic, as compared to the other DAergic systems (Thierry et al., 1976; Blanc et al., 1980; Deutch et al., 1985; Jedema and Moghaddam, 1994; Cuadra et al., 1999; Bassareo et al, 2002). DA in the PFCX is responsive to salient stimuli, such as novelty, food and food conditioned stimuli (Feenstra and Botterblom, 1996; Bassareo and Di Chiara, 1997; Bassareo et al., 2002; 2007).

2. Aims

Microdialysis, voltammetry and electrophysiological studies have shown that NAc shell DA and DA neurons projecting to the shell are preferentially activated by drugs of abuse after response non-contingent (passive) as well as response-contingent exposure (Pontieri et al. 1995, 1996; Tanda et al. 1997; Aragona et al., 2008; Lecca et al, 2006a; 2006b; 2006c; 2007a; 2007b). Feeding after non-contingent presentation of palatable food stimulates in vivo DA transmission in the NAc shell and core and in the medial prefrontal cortex (PFCX). This response, however, undergoes habituation after a single trial specifically in the shell (Bassareo and Di Chiara, 1997; 1999a; 1999b; Bassareo et al., 2003; Gambarana et al, 2003; Rada et al, 2005; Danielli et al, 2009). Habituation lasts for at least 24 hours and fully recovers within 5 days (Bassareo and Di Chiara, 1997). In contrast to the shell, NAc core DA transmission is potentiated by non-contingent single-trial exposure to palatable food (Bassareo and Di Chiara, 1997), consistently with the notion that DA plays different functions in the two NAc subdivisions.

Habituation of in vivo stimulation of DA transmission in the NAc shell by palatable food has been interpreted to indicate that NAc shell DA plays a role in the learning process by which pavlovian stimuli acquire the ability to act as conditioned incentives thus promoting approach to rewards and reward-predicting stimuli and strengthening instrumental responding (pavlovian to instrumental transfer).

In contrast to palatable food, drugs of abuse do not induce habituation of the responsiveness of NAc shell DA transmission after repeated non-contingent as well as contingent exposure (Bassareo et al, 2003; Cadoni and Di Chiara, 1999; Lecca et al, 2006a; 2006b; 2006c; 2007a; 2007b). Thus, given the role assigned to NAc shell DA in incentive learning, it has been speculated that the failure of NAc shell DA transmission to habituate after repeated exposure might abnormally strengthen learning of drug-conditioned incentives, thus contributing to the dependence liability of drugs of abuse.

Further studies from our laboratory have shown that response contingency drastically affects the responsiveness of DA transmission in the two subdivisions of the NAc under repeated drug exposure. Thus, while repeated non-contingent exposure to heroin and cocaine reduces the DA responsiveness in the NAc shell DA and sensitizes it in the core, response-contingent exposure to heroin and cocaine does not change the preferential pattern of response in the shell observed after acute drug exposure (Cadoni and Di Chiara, 1999; Lecca et al, 2006a; 2006b; 2006c; 2007a; 2007b).

As far as food is concerned, although various studies are available comparing shell versus core DA transmission during operant and free food consumption, none has directly tested this issue.

Microdialysis studies either did not find differences between shell and core in the changes of DA during responding for food (Ostlund et al, 2011; Chen and Feenstra, 2006) or the differences obtained did not occur during responding but after it (Sokolowsky et al, 1999). It has been even reported that no changes in dialysate DA take place in the NAc shell and core of rats responding for food on a FR1 schedule (Segovia et al, 2011). As far as voltammetric studies are concerned two problems arise. The first problem is the difficulty of comparing voltammetric and microdialysis studies due to basic differences in the aspects of DA transmission estimated by the two methodologies. It has been suggested that while microdialysis estimates the tonic mode of DA transmission, related to single spike firing, voltammetry estimates its phasic modality, resulting from burst firing (Grace, 2000). We rather believe that both microdialysis and voltammetry estimate extracellular DA mainly arising from burst firing their differences being related to the fact that while microdialysis averages phasic DA changes over a time frame of minutes and expresses them in relation to (as % of) absolute basal values, voltammetry estimates phasic changes from a zero baseline over a subsecond (100ms) time frame. The second problem is related to the large discrepancies between existing studies. For example, Cacciapaglia et al (2012) reported that presentation of visual-auditory cues signaling reward availability elicits a phasic increase of extracellular DA in the NAc shell and core that fades within 2 seconds, when the lever is extended into the chamber and response is emitted to obtain the reward. This cue-related response is larger in the NAc shell than in the core and is followed by a second response that takes place immediately after lever extension and selectively in the NAc shell DA. This second component of the DA change is lower and slower and coincides with sucrose reward, extending in some rats over 10 sec after cue presentation. Quite in contrast with the above study, Brown et al (2011) observed phasic activation of DA in the NAc core but no change in the shell in relation to the presentation of discriminative cues signalling food availability. In turn while Brown et al (2011) report an increase of phasic DA in the NAc core but not in the shell in response to food, Roitman et al (2008) and Wheeler et al (2011) found just the opposite.

Given these large discrepancies of the literature, we intended to re-examine in our laboratory the responsiveness of DA transmission in various DA terminal areas to responding for sucrose. Therefore, we monitored by microdialysis the changes in dialysate DA in the NAc shell and core and medial prefrontal cortex (mPFCX) of rats trained to respond for sucrose on two different fixed-ratio schedules (FR1 and FR5) and tested during responding for sucrose, response non-contingent sucrose presentation and under extinction conditions, i.e. in the presence of all the stimuli generated during the operant sessions, except for sucrose.

To this end rats were trained to self-administer sucrose pellets and dialysate DA was

monitored in the NAc shell, core and mPFCX in different groups of rats under three different conditions: response-contingent (active) presentation of sucrose; extinction in the presence of discriminative cues associated to sucrose availability; response non-contingent presentation of sucrose. Different groups of rats were trained on FR1 and FR5 schedule respectively and after full training DA was monitored in the NAc shell, core and mPFCX every day for three days under the above conditions. In another series of rats DA was monitored for two weeks in the NAc shell and core during acquisition of responding for sucrose and on the last three days they were tested under responding for sucrose, extinction and passive sucrose presentation.

3. Materials & Methods

3.1 Subjects

Male Sprague-Dawley rats (250-275 g, Harlan, Udine, Italy) were housed in groups of six in Plexiglas cages, under constant temperature (23 C°) and humidity (60%) and a12 h light/dark cycle (light from 8 a.m. to 8 p.m.), with standard food (MIL topi e ratti, GLP diets, Stefano Morini, S. Polo D'Enza, RE, Italy) and water ad libitum.

All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Economic Commission (EEC Council 86/609; DL: 27.01.1992, N° 116) and with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003).

3.2 Surgery

Rats were anaesthetized with 400 mg/Kg i.p. of chloral hydrate (Carlo Erba, Milano, Italy). A guide cannula (Fig. 1) (Plasticone, Roanoke, VA, USA) was stereotaxically implanted under the following coordinates: NAc shell (A: 2.0; L: 1 from bregma, V: -3.6 from dura), NAc core (A: 1.6.0; L: 1.9 from bregma, V: -3.4 from dura), mPFCX (A: 3.7; L: 0.8 from bregma; V: -2.0 from dura), according to the atlas of Paxinos & Watson (1998). Guide cannulae were plugged with a dummy cannula.

After surgery, rats were housed in individual cages (45x21x24 cm) under the same conditions mentioned above. Rats were left to recover for 10 days and during the first 5 days were administered with Gentamicin sulphate (40 mg/Kg s.c.). Rats were manipulated once a day for 5 minutes during the whole training period.

After recovery rats were fed with 15g of standard food (MIL topi e ratti, GLP diets, Stefano Morini, S. Polo D'Enza, RE, Italy) in order to keep their weight around 90% of their ad libitum weight. Water was ad libitum for the whole duration of the experiments.

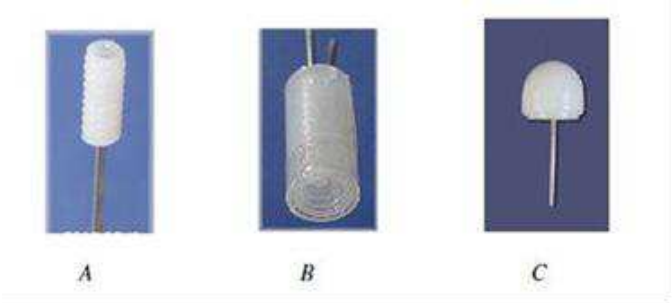


Figure 1: A) guide cannula, B) push-pull, C) dummy-cannula

3.3 Microdialysis

3.3.1 Probe construction

Microdialysis probes were prepared according to the method described by Lecca et al. (2006 a and b) using a membrane (AN 69, Hospal Dasco, Italy) made-up of a sodium-meta-allyl-sulfonate acrylic copolymer, with an external diameter of 310 µm and an internal diameter of 220 µm.

For each experimental session a new probe was inserted into the guide cannula.

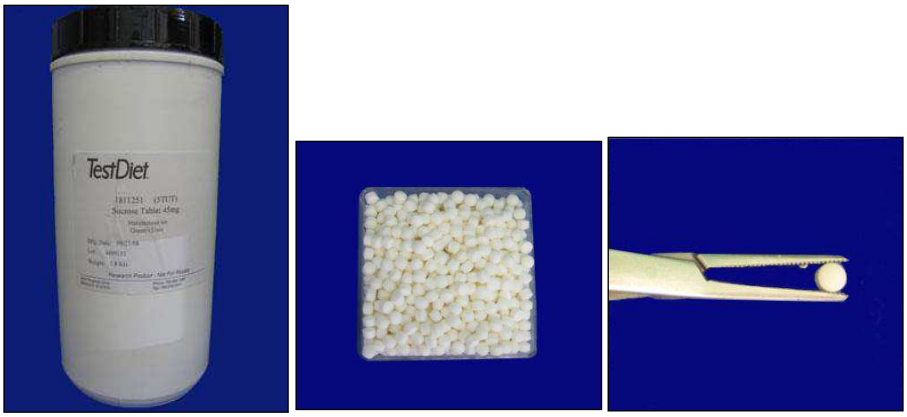
Figure 2: chronic microdialysis probe.

3.3.2 Microdialysis experiments

At the beginning of each microdialysis session, the microdialysis probes were connected to an infusion pump and perfused with a Ringer's solution (147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$) at a constant rate of 1 µl/min and were inserted through the guide cannula in the animal brain. Dialysate samples (5 µl) were taken and analysed every 5 min and were injected without purification into a high-performance liquid chromatograph (HPLC) 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) to quantify DA. The first electrode of the detector was set at + 125 mV (oxidation) and the second at - 175 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). With these conditions the sensitivity of the assay for DA was 5 fmoles/sample.

3.4 Sucrose

Sucrose pellets of 45 mg each were utilized during the sessions (Test Diet, 1050 Progress Drive Richmond, IN 47374).



A B C

Figure 3: Sucrose pellets (Test Diet, 1050 Progress Drive Richmond, IN 47374)

3.5 Passive sucrose pellet presentation

In order to investigate if repeated feeding of sucrose induces habituation of feeding-induced increase of dialysate DA in the NAc shell, rats naïve to sucrose and implanted with microdialysis probes were placed in the Skinner boxes and dialysate samples were taken every 5 min. After stabilization of basal dialysate DA, 40 sucrose pellets were delivered in the food dispenser in 10 min. The test was repeated two more times on each of the next two days.

3.6 Operant training

After ten days of recovery from surgery, each one of the groups of rats implanted in the NAc shell, core and mPFCX was divided into two groups and one group was trained daily, except weekends, on FR1 for two weeks and the other on FR1 on the first week, on FR3 on the second and on FR5 on the third.

Sessions lasted one hour and took place between 9 and 14 a.m. in acoustically isolated and ventilated operant cages (Coulbourn Instruments, Allentown, NJ USA).

The two nose-pokes holes were placed on one wall, 2 cm from the cage's floor. The active nose-poke was illuminated with a green-yellow light while the inactive one was illuminated by a red light.

Between the two nose-pokes holes was placed the food dispenser. In the same wall was placed a loud-speaker producing a tone with a frequency of 4500Hz.

The number of nose-pokes made and of rewards earned were recorded by Graphic State 2 software, Coulbourn Instruments, USA.

Each one hour session was composed by a cyclic alternation of 3 phases:

1) Phase 1, lasting 15 s during which the house light and the nose poke lights were turned on and a tone was activated to signal reward availability. Animals could obtain the reward only by nose-poking in the active hole. Failure to respond correctly resulted in the switch off of visual and auditory cues and switch to phase 3 without going into phase 2.

2) Phase 2: a sucrose pellet was dropped into the food dispenser and after 5 s the next phase was initiated.

3) Phase 3: all cues were turned off and reward was not available for a fixed interval of 7 sec in the case of the FR1 schedule and for a variable interval (random, with a mean of 7s) in the case of FR5.

3.7 Microdialysis in trained rats

After completion of the operant training, all rats were tested on three microdialysis experiments performed one on each day, on three consecutive days.

The three microdialysis experiments consisted of

1) a session of responding for sucrose under the same schedule and conditions under which the rats had been trained

2) a session of responding under extinction using the same schedule utilized for training

3) a session of passive presentation of sucrose pellets throughout the food dispenser at the same mean rate at which rats earn them during the operant sessions but in the absence of the discriminative cues signalling sucrose availability.

3.8 Microdialysis during training on FR1 responding for sucrose

In a separate group of rats dialysate DA was monitored within subjects during training of FR1 responding for sucrose. After recovery from surgery, animals started the sucrose self-administration training, under the same conditions described above. Microdialysis was monitored during responding for sucrose, every two days and for two weeks, apart from weekends, for a total of 10 sessions. Starting on the first day of the third week three different microdialysis experiments were performed on three consecutive days, one on each day, under responding for sucrose, under extinction and under non-contingent sucrose presentation.

3.9 Histology

At the end of the experiment, probes were removed and animals were anaesthetized with 400 mg/Kg i.p. of chloral hydrate and then their brain was removed. The brains were kept in a 4%

formaldehyde solution for at least one week and successively they were cut on a vibratome in serial coronal slices oriented according to the atlas of Paxinos & Watson (1998). The location of the probes was reconstructed and referred to the atlas of Paxinos & Watson (1998) (Fig. 4).

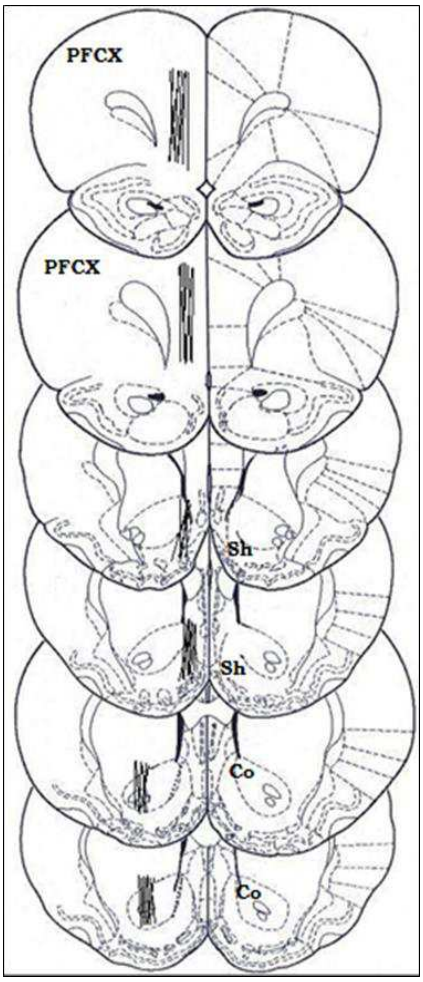


Figure 4: Schematic drawing of the localization of dialysis probes (dialysis portion) in the PFCX, NAc shell and core compartments (PFCX, Prefrontal Cortex; Co, NAc core; Sh, NAc shell Reconstructed from Paxinos & Watson, 2007)

3.10 Statistics

Statistical analysis was carried out by Statistica for Windows. Depending on the experiments, data were analysed by one-, two- or three-way ANOVA. Results from treatments showing significant overall changes were subjected to post hoc Tukey's test; $p<0.05$ was taken as significant. Basal values were the means of three consecutive samples differing by no more than 10%. Microdialysis data were expressed as percentage of basal values. Regression analysis of the relationship between DA levels in the NAc shell and core and PFCX and nose poking activity was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, CA, USA).

4. Results

4.1 Habituation of NAc shell dopamine to sucrose feeding

Habituation of DA responsiveness to feeding of palatable foods has been shown by us for a salty food like (Fonzies®) as well as for a sweet food (Kinder®) (Bassareo et al. 1997, Bassareo and Di Chiara 1999b). In order to establish if habituation also applies to sucrose, dialysate DA was monitored every day for three days during feeding of experimenter-administered sucrose pellets in rats that had never been previously exposed to sucrose.

Figure 5 shows the time-course of dialysate DA in the NAc shell on three successive daily sucrose-feeding trials.

Two-way ANOVA showed an effect of day ($F_{2,13}$=17.28; p<0.01), time ($F_{12,156}$=12.64; p<0.01) and a day x time interaction ($F_{24,156}$=5.44; p<=0.01). Tukey's test showed that DA increased DA only on the first day.

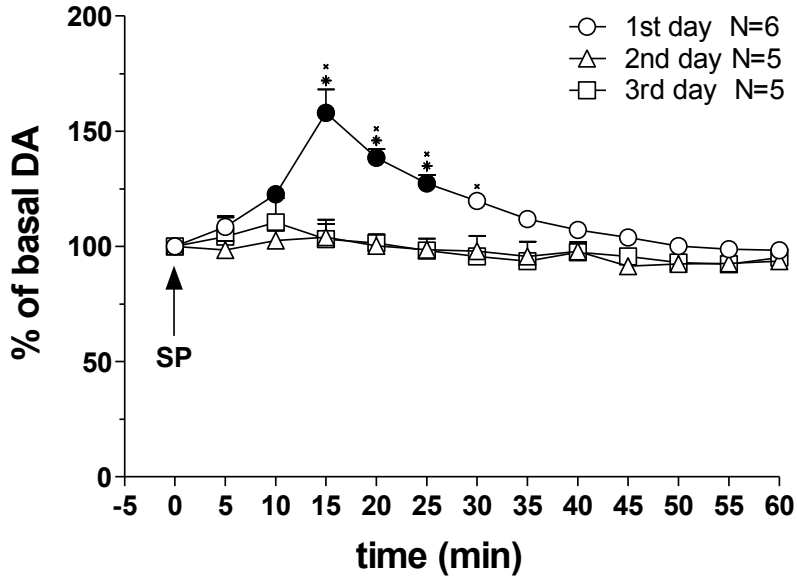


Figure 5: Time-course of dialysate DA in the NAc shell during passive sucrose pellet feeding.
Basal values of NAc shell DA (means±SEM) were 27±3 fmoles (N=16).
Data are means±SEM of the results obtained in 16 rats.
Filled symbols: p<0.05 vs basal values;
*: p<0.05 vs the 2nd day;
x: p<0.05 vs the 3rd day.

4.2 Monitoring dialysate dopamine in rats trained on FR1 and FR5 responding for sucrose

4.2.1 Rats trained on FR1

4.2.1.1 Acquisition of FR1 responding for sucrose

Figure 6 shows the average number of cumulative active and inactive nose pokes performed by rats during training of FR1 responding for sucrose. Active responding reached the asymptote on the 7th day in the three groups, indicative of full training.

Three way ANOVA showed a main effect of nose-poke (active versus passive) ($F_{1,38}$= 233,90; $p<0.01$), area ($F_{2,38}$= 8,98; $p<0.01$) and day ($F_{9,342}$=30,07; $p<0.01$) and an interaction of nose-poke x area ($F_{2,38}$=10.35; $p<0.01$) and nose-poke x day ($F_{9,342}$=26.26; $p<0.01$).

Post hoc test showed a higher number of active nose pokes during training in rats implanted in the mPFCX compared to rats implanted in the shell and in the core that in turn were not different.

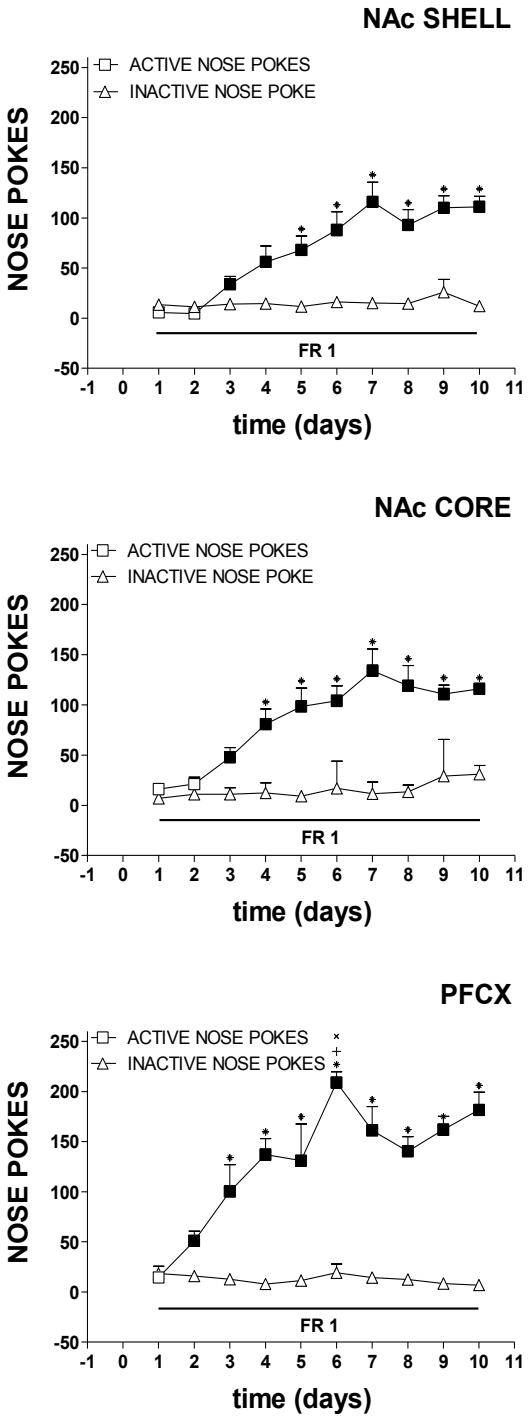


Figure 6: Cumulative active (squares) and inactive (triangles) nose-pokes during the sucrose SA training period (FR 1 schedule).
Data are means±SEM of the results obtained in 9 rats for NAc shell, 12 rats for NAc core and 7 rats for mPFCX.
Filled symbols, $p<0.05$ vs 1st day;
*, $p<0.05$ vs inactive nose pokes;
x, $p<0.05$ vs active nose pokes shell group;
+, $p<0.05$ vs active nose pokes core group.

4.2.2 NAc shell and core dopamine microdialysis in rats trained on FR1

4.2.2.1 Responding for sucrose

Figure 7 (A) shows the time-course of dialysate DA in the NAc shell and core and of active nose-pokes during FR1 responding for sucrose.

Two-way ANOVA showed an effect of area ($F_{1,18}$=15.83; p<0.01), time ($F_{6,108}$=7.77; p<0.01) and an area x time interaction ($F_{6,108}$=4.79; p<=0.01). Post-hoc test showed an increase of dialysate DA in the NAc shell but not in the core.

Active nose-pokes were high for 30 min.

As shown in figure 8, a significant correlation between percent of DA levels and nose poking with r=0.53 and a significant slope (p<0.01) was obtained in the NAc shell but not in the NAc core (r=0.12; slope: p=0.17 N.S.). The two slopes are statistically different ($F_{1,236}$=20.63, p<0.0001).

4.2.2.2 Responding under extinction

Figure 7 (B) shows the time-course of dialysate DA in the NAc shell and core and of active nose-pokes under extinction in the presence of cues signalling sucrose availability.

Two-way ANOVA showed an effect of area ($F_{1,14}$=8.46; p=0.011), time ($F_{6,84}$=11.29; p<0.01) and an interaction area x time ($F_{6,84}$=9.75; p<0.01). Post-hoc test showed that DA increased in the NAc shell but not in the core.

As shown in figure 9, a significant correlation between percent of DA levels and nose poking with r=0.51 and a significant slope (p<0.01) was obtained in the NAc shell, but not in the NAc core (r=0.18; slope: p=0.052 N.S.). The two slopes are statistically different ($F_{1,188}$=20.75, p<0.0001).

4.2.2.3 Response to non-contingent sucrose feeding

Figure 7 (C) shows the time-course of DA in the NAc shell and core during non-contingent sucrose presentation and feeding. Bars show the number of pellets presented every 5 minutes.

Two-way ANOVA showed an effect of area ($F_{1,6}$=4.46; p<0.01), time ($F_{8,48}$=10.61; p<0.01) and an interaction area x time ($F_{8,48}$=2.0; p<0.01).Post-hoc test showed an increase of DA both in the shell and in the core.

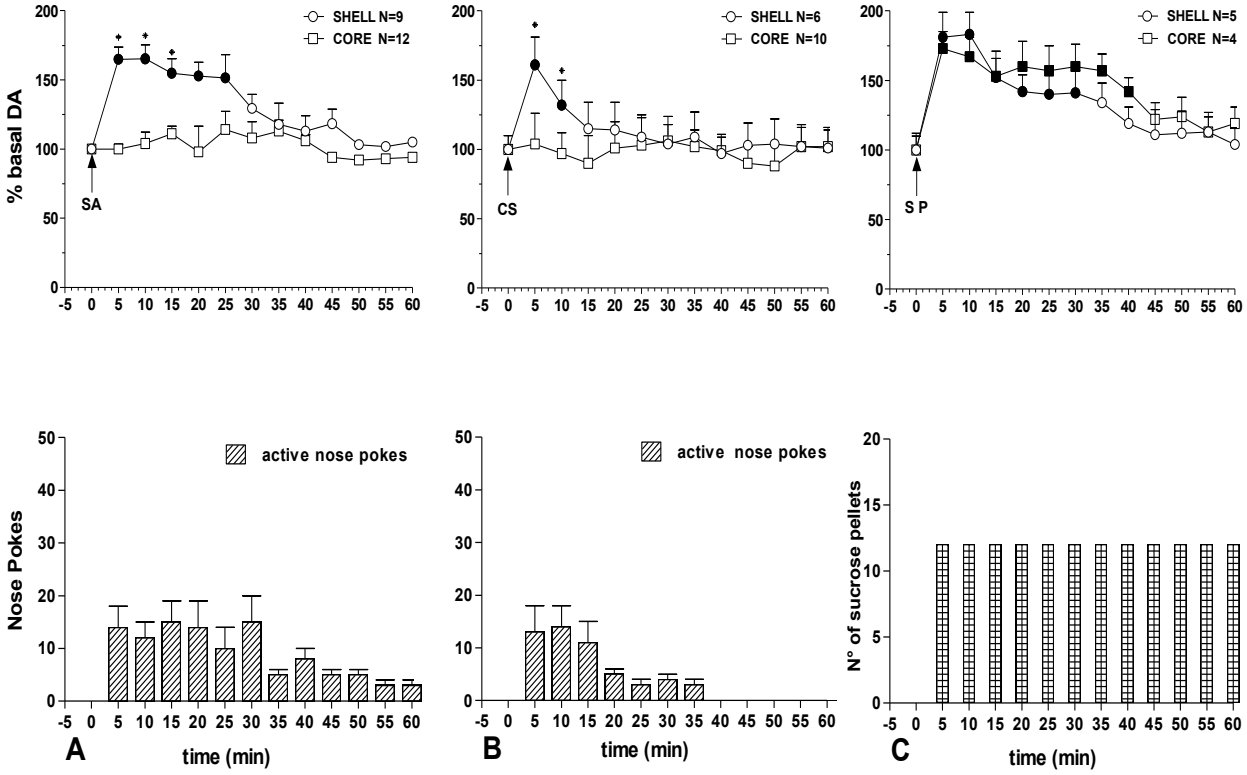


Figure 7: Time-course of dialysate DA in the NAc shell (circles) and core (squares) and active nose pokes or pellets (bars, means of shell and core group or number of pellets presented every 5 min) under FR1 responding for sucrose (A), extinction (B) and non-contingent sucrose pellet presentation (C).Group dialysed after FR1 training.

Basal values of DA (fmoles means±SEM) NAc shell 25±3 (N=20), core 26±4 (N=26).

Data are means±SEM. of the results obtained in the number of rats indicated in the figure.

Filled symbols: $p<0.05$ vs basal values;

*: $p<0.05$ vs values obtained in the core.

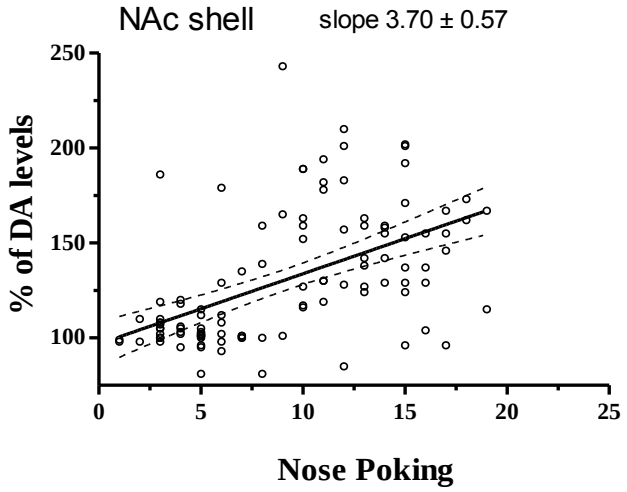


Figure 8: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose. Group dialysed after FR1 training.

Graph shows the correlation between the DA output in the NAc shell (N=9) (Y-axis) and nose poking (X-axis) during FR1 sucrose feeding. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

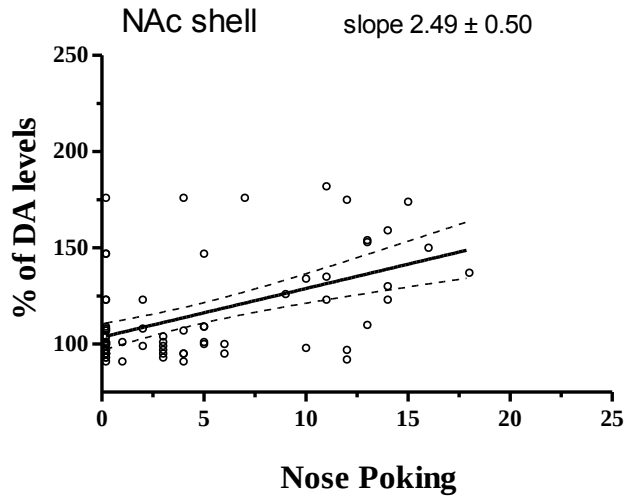


Figure 9: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during extinction. Group dialysed after FR1 training.
Graph shows the correlation between the DA output in the NAc shell (N=6) (Y-axis) and nose poking (X-axis) during extinction. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session

4.2.3 mPFCX dopamine microdialysis in rats trained on FR1

4.2.3.1 Responding for sucrose

Figure 10 (A) shows the time course of DA in the mPFCX during sucrose pellets self-administration under FR1 schedule and relative nose pokes (bars).

One-way ANOVA showed an effect of time ($F_{6,30}=15.66$; $p<0.01$).

Tukey's test showed an increase of DA with respect to basal value. Active nose-pokes were high for 30 min.

As shown in figure 11, a significant correlation between percent of DA levels and nose poking with $r=0.54$ and a significant slope ($p<0.01$) was obtained in the mPFCX.

4.2.3.2 Responding under extinction

Figure 10 (B) shows the time-course of dialysate DA in the mPFCX and of active nose-pokes under extinction and in the presence of cues signalling sucrose availability.

One-way ANOVA showed an effect of time ($F_{6,30}=19.32$; $p<0.01$). Tukey's test showed increased dialysate of DA in the mPFCX.

As shown in figure 12, a significant correlation between percent of DA levels and nose poking with $r=0.73$ and a significant slope ($p<0.01$) was obtained in the mPFCX.

4.2.3.3 Response to non-contingent sucrose feeding

Figure 10 (C) shows the time-course of DA in the mPFCX during the sucrose pellets passive presentation. Bars indicate the number of pellets presented every 5 minutes.

One-way ANOVA showed an effect of area x time ($F_{6,18}$=12.40; p<0.01). Tukey's test confirmed an increase of DA in mPFCX compared to basal value

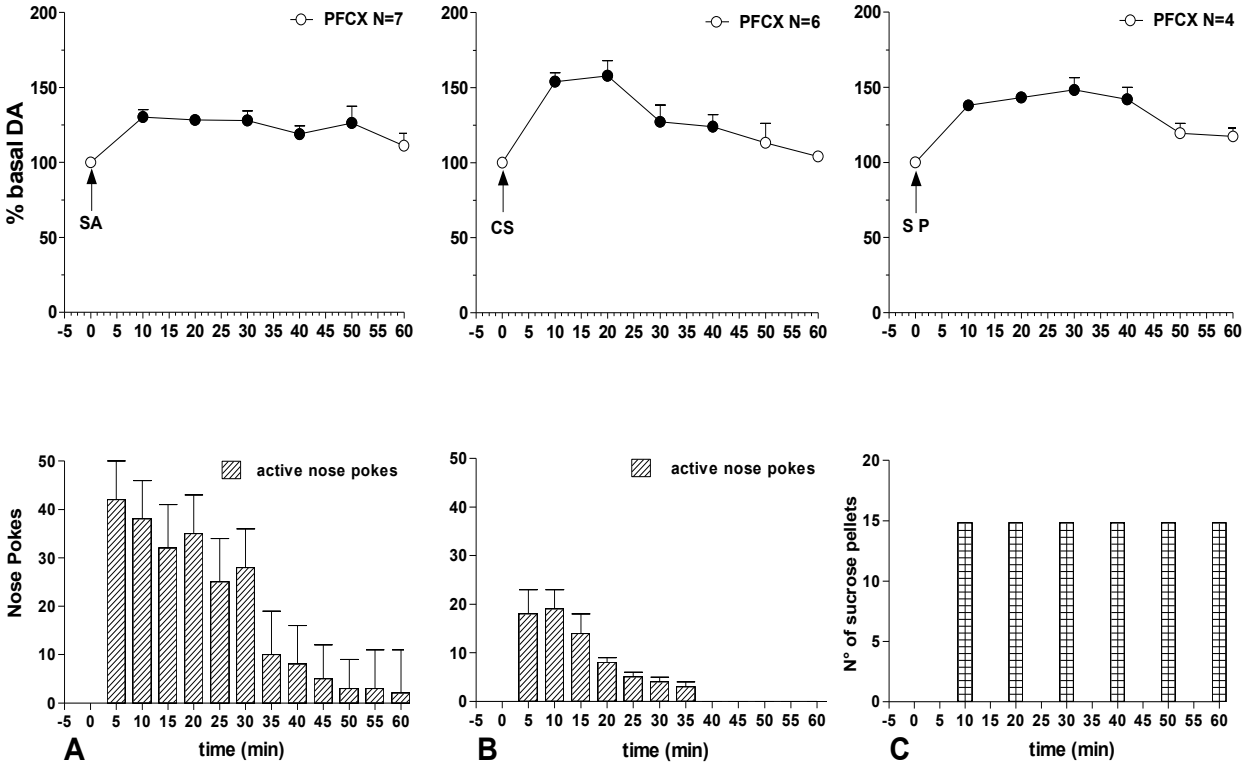


Figure 10: Time-course of dialysate DA in the mPFCX and of active nose pokes / mean pellets presented (bars) under FR1 responding for sucrose (A), extinction (B) and non-contingent sucrose pellet presentation (C). Group dialysed after FR1 training.

Basal values of DA (means±SEM): 10±1 fmoles (N=17).

Data are means±SEM. of the results obtained in the number of rats indicated in the figure.

Filled symbols: p<0.05 vs basal values.

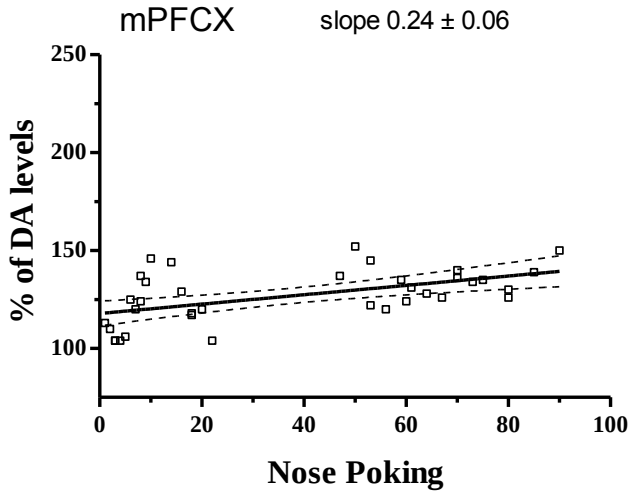


Figure 11: Regression analysis of the relationship between increase in DA levels in the mPFCX and nose poking activity during FR1 responding for sucrose. Group dialysed after FR1 training.
Graph shows the correlation between the DA output in the mPFCX (N=7) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session

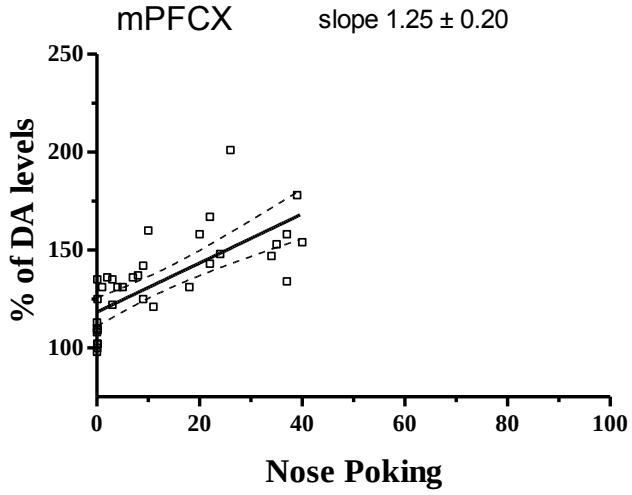


Figure 12: Regression analysis of the relationship between increase in DA levels in the mPFCX and nose poking activity during extinction. Group dialysed after FR1 training.
Graph shows the correlation between the DA output in the mPFCX (N=6) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session

4.2.4 Responding for sucrose during FR5 training

Figure 13 shows the average number of cumulative active and inactive nose-pokes performed by rats during the sucrose SA training.

Three way ANOVA of data obtained during the last 7 days of training with a FR5 schedule showed a main effect of nose poke (active versus passive) ($F_{1,72}$=296,79; $p<0.01$). Post hoc analysis showed that during FR5 training the number of active nose-pokes increased up to a maximum that

was not different for three consecutive sessions. No significant differences were obtained between shell and core but responding was higher in rats implanted in the mPFCX as compared to those implanted in the NAc shell and in the core.

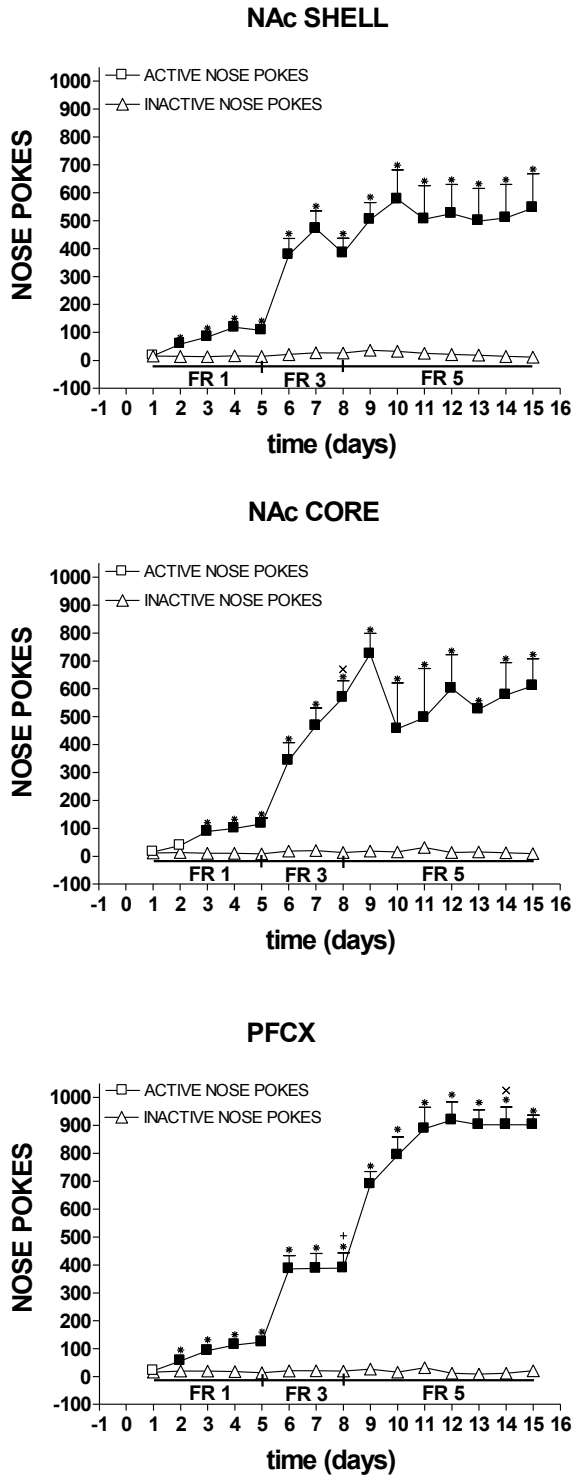


Figure 13 Cumulative active (squares) and inactive (triangles) nose-pokes during training of responding for sucrose.
Data are means±SEM of the results obtained in 9 rats for NAc shell, 12 rats for NAc core and 7 rats for mPFCX. Filled symbols, $p<0.05$ vs 1st day; *, $p<0.05$ vs inactive nose pokes; x, $p<0.05$ vs active nose pokes shell group; +, $p<0.05$ vs active nose pokes core group.

4.2.5 NAc shell and core dopamine microdialysis in rats trained on FR5

4.2.5.1 Responding for sucrose

Figure 14 (A) shows the time-course of dialysate DA from the NAc shell and core and of active nose-pokes during FR5 responding for sucrose.

Two-way ANOVA showed an effect of area ($F_{1,13}$=32.02; $p<0.01$), time ($F_{8,104}$=3.67; $p<0.01$) and an interaction area x time ($F_{8,104}$=2.76; $p<0.01$). Tukey's test showed an increase of DA in the shell.

As shown in figure 15, a significant correlation between percent of DA levels and nose poking with r=0.36 and a significant slope ($p<0.01$) was obtained in the NAc shell, but not in the NAc core (r=-0.42; slope: p=0.75 NS). The two slopes are statistically different ($F_{1,176}$=29.56, $p<0.0001$).

4.2.5.2 Responding under extinction

Figure 14 (B) shows the time-course of dialysate DA in the NAc shell and core and of active nose-pokes under extinction.

Two-way ANOVA showed an effect of time ($F_{5,55}$=7.07; $p<0.01$). Tukey's test showed a strengthening of DA both in the shell and in the core of the NAc.

As shown in figure 16, a significant correlation between percent of DA levels and nose poking with r=0.52 and a significant slope ($p<0.01$) was obtained in the NAc shell, but not in the NAc core (r=0.22; slope: p=0.062 NS). The two slopes are statistically different ($F_{1,152}$=8.53, p=0.004).

4.2.5.3 Response to non-contingent sucrose feeding

Figure 14 (C) shows the time-course of DA in the NAc shell and core. The bars indicate the number of pellets presented every 5 minutes.

Two-way ANOVA showed an effect of time ($F_{9,63}$=9.94; $p<0.01$) and an interaction of area x time ($F_{9,63}$=12.28; $p<0.01$). Tukey's test showed an increase of DA both in the shell and in the core.

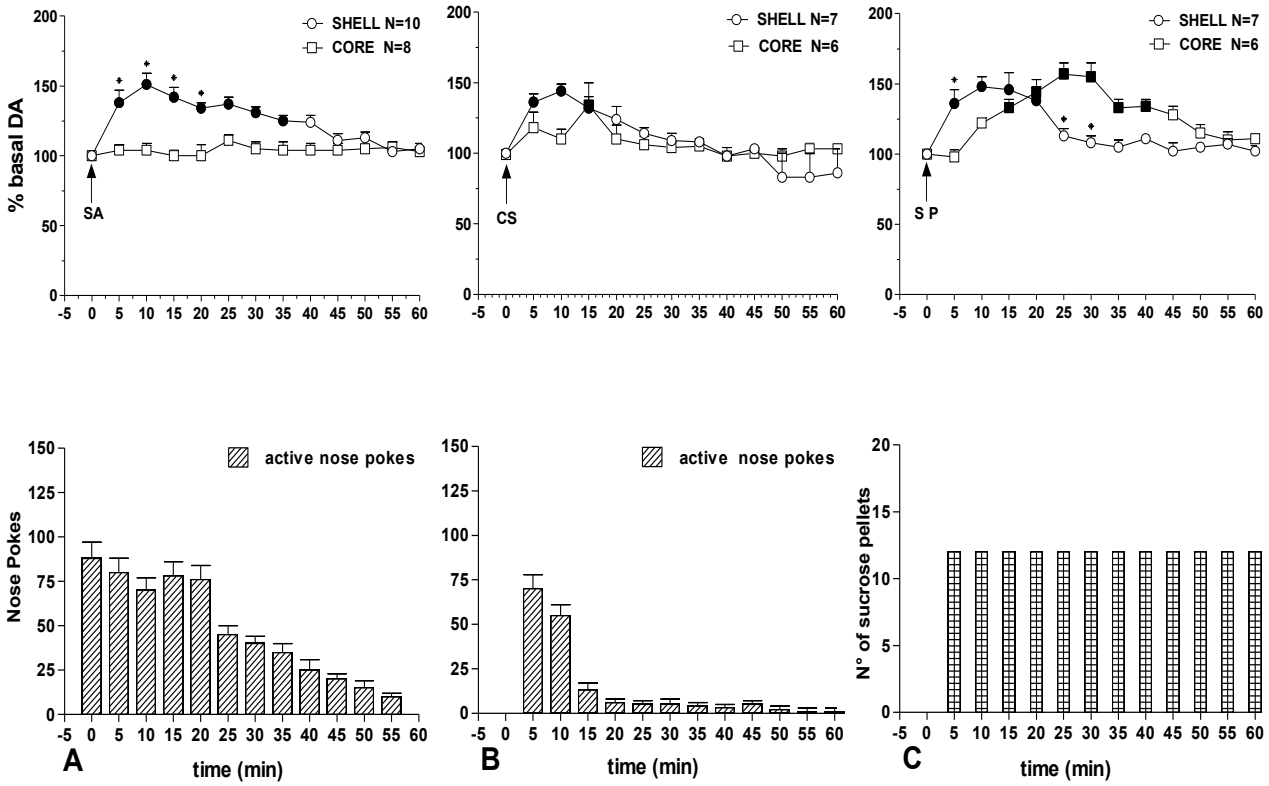


Figure 14: Time-course of dialysate DA in the NAc shell (circles) and core (squares) and active nose pokes (bars, means of shell and core group or number of pellets presented every 5 min.), during FR5 responding for sucrose (A), extinction (B) and after non-contingent sucrose pellets presentation(C). Group dialysed after FR5 training.

Basal values of DA (means±SEM) in 5-min samples were as follow: NAc shell 27±3 fmoles (N=24), core 25±3 fmoles (N=20). Data are means±SEM. of the results obtained in the number of rats indicated in the figure. Filled symbols: $p<0.05$ vs basal values; *: $p<0.05$ with respect to values obtained in the core.

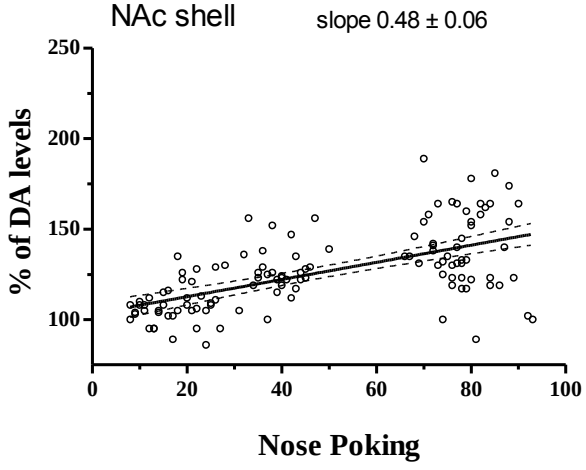


Figure 15: Regression analysis of the relationship between increase in DA levels in the NAc shell nose poking activity during FR1 responding for sucrose. Group dialysed after FR5 training.

Graph shows the correlation between the increase of DA output in the NAc shell (N=10) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

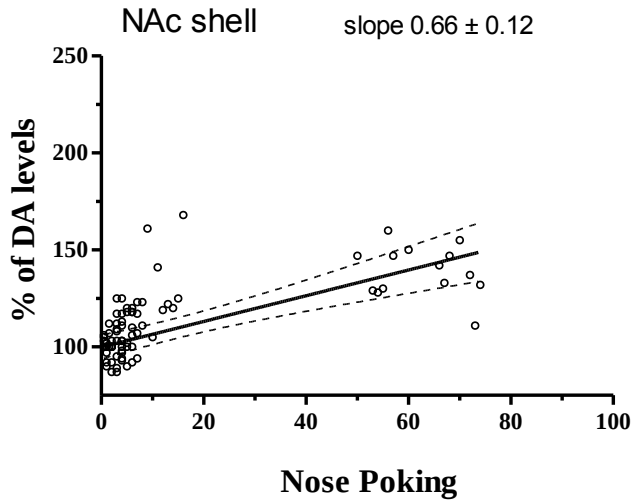


Figure 16: Regression analysis of the relationship between increase in DA levels in the NAc shell and nose poking activity during extinction. Group dialysed after FR5 training.
Graph shows the correlation between the increase of DA output in the NAc shell (N=6) (Y-axis) and nose poking (X-axis) during extinction session. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session

4.2.6 mPFCX dopamine microdialysis in rats trained on FR5

4.2.6.1 Responding for sucrose

Figure 17 (A) shows the time-course of dialysate DA in the mPFCX and active nose-pokes during FR5 responding for sucrose.

One-way ANOVA showed an effect of time ($F_{6,48}$=11.24; p<=0.05).Tukey's test showed an increase of DA from basal values.

The correlation between percent of DA levels and nose poking in rats implanted in the mPFCX it is not significant (r=-0.17; slope: p=0.27 N.S.).

4.2.6.2 Responding under extinction

Figure 17 (B) shows the time-course of dialysate DA in the mPFCX and nose-poking activity resulting in cues signalling sucrose availability during conditioned stimuli presentation (tone and lights previously associated with sucrose pellets administration).

One-way ANOVA did not show any effect ($F_{6,48}$=0.98; p=0.45) Active nose pokes were high for 10 min. The correlation between percent of DA levels and nose poking in rats implanted in the mPFCX it is not significant (r=-0.16; slope: p=0.24 N.S.).

4.2.6.3 Response to non-contingent sucrose feeding

Figure 17 (C) shows the time-course of DA in the mPFCX during the sucrose pellets passive administration by the operator. Bars indicate the number of pellets presented every 5 minutes.

One-way ANOVA showed an effect of time ($F_{6,30}$=4.07; p<=0.05).Tukey's test confirmed an increase of DA in mPFCX compared to basal value.

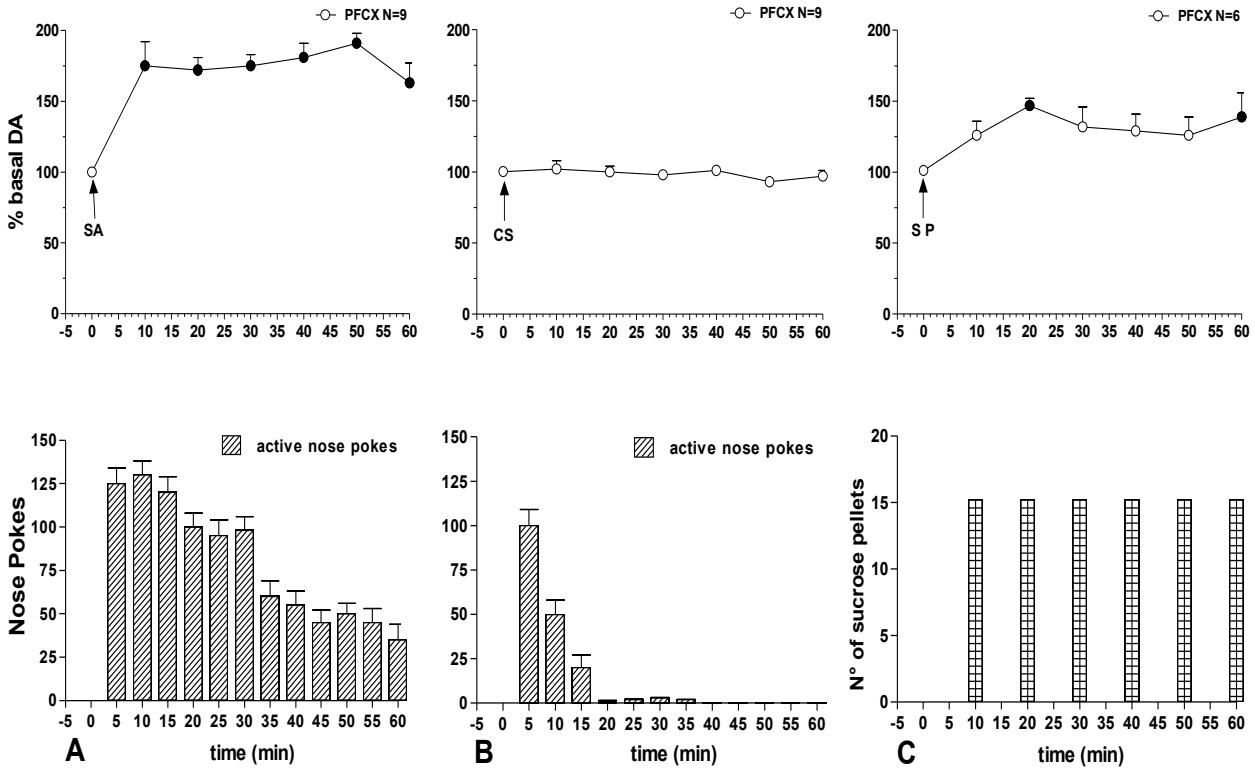


Figure 17: Time-course of dialysate DA in the mPFCX (circles) and active nose pokes or number of pellets presented (bars) during responding for sucrose (A), extinction (B) and during non-contingent sucrose pellet presentation(C). Group dialysed after FR5 training.
Basal values of DA (means±SEM) in 5-min samples were 11±1 fmoles (N=24).
Data are means±SEM. of the results obtained in the number of rats indicated in the figure.
Filled symbols: p<0.05 vs basal values.

4.3 Monitoring dialysate dopamine during FR1 training

4.3.1 Responding for sucrose during training

Figure 18 shows the average number of cumulative active and inactive nose-pokes performed by rats during the sucrose SA acquisition.

Three way ANOVA showed main effects of nose poke ($F_{1,22}$= 141.98; p<0.01), day ($F_{9,198}$=20.96; p<0.01), nose poke x day interaction ($F_{9,198}$=20.69; p<0.01). Post hoc analysis showed that during the sucrose SA acquisition the number of active nose-pokes increased every day still a maximum

(plateau) and showed that the active nose-pokes were more than inactive nose-pokes. Post hoc test also did not show difference between shell and core group.

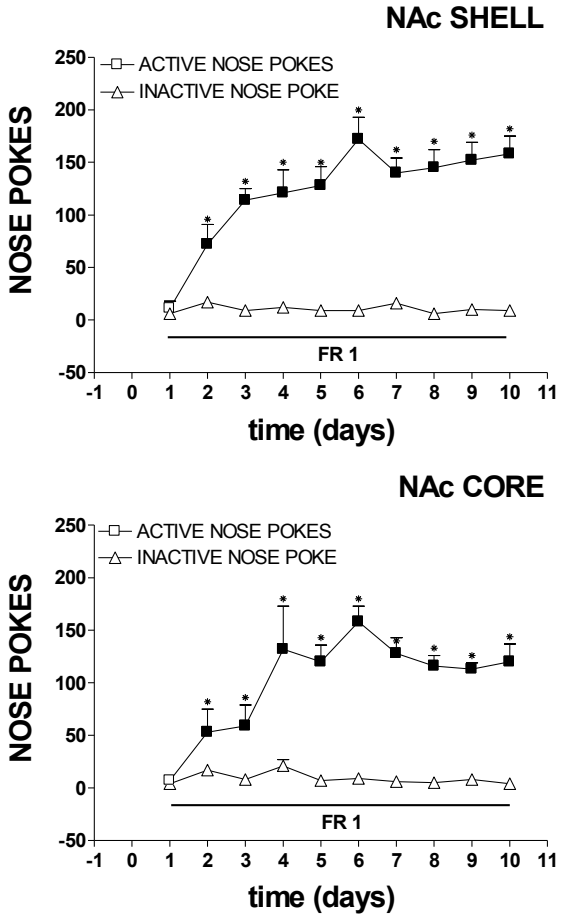


Figure 18: Cumulative active (squares) and inactive (triangles) nose-pokes during FR1 training of responding for sucrose in rats microdialyzed every second day.
Data are means±SEM of the results obtained in 7 rats for NAc shell and 6 rats for NAc core.
Filled symbols, $p<0.05$ vs 1st day;
*, $p<0.05$ vs inactive nose pokes.

4.3.2 NAc shell and core dopamine microdialysis during training on FR1 responding for sucrose

Figure 19 shows the time-course of dialysate DA during training on FR1 responding for sucrose on each session

1st session: Two-way ANOVA did not show an increase of DA in both areas ($F_{area1,11}=0.344$; $p=0.57$; $F_{time12,132}=1.78$; $p=0.06$; $F_{areaxtime12,132}=0.58$; $p=0.86$) (figure 19.A).

The correlation between percent of DA levels and nose poking is not significant in rats implanted in the NAc shell (r=-0.18; slope: p=0.13 N.S.) and in rats implanted in the NAc core (r=-0.13; slope: p=0.25 N.S.)

3rd session: Two-way ANOVA showed an effect of time ($F_{12,120}$=5.26; p=0.00001). Post hoc analysis showed a selective increase of DA in the NAc shell (figure 19.B).

As shown in figure 21, a significant correlation between percent of DA levels and nose poking with r=-0.36 and a significant slope (p<0.01) was obtained in the NAc shell, but not in the NAc core (r=0.17; slope: p=0.16 NS). The two slopes are not statistically different ($F_{1,140}$=1.5, p=0.22).

5th session: Two-way ANOVA showed an effect of time ($F_{12,120}$=18.03; p=0.00001) and an interaction area x time ($F_{12,120}$=2.78; p=0.002). Post hoc analysis showed a selective increase of DA in the NAc shell. (figure 19.C).

As shown in figure 22, a significant correlation between percent of DA levels and nose poking with r=-0.52 and a significant slope (p<0.01) was obtained in the NAc shell and in the NAc core (r=0.34; slope: p<0.01). The two slopes are not statistically different ($F_{1,140}$=0.76, p=0.38).

6th session: Two-way ANOVA showed an effect of area ($F_{1,9}$=18.41; p=0.002), of time ($F_{12,108}$=5.05; p<0.01) and an interaction area x time ($F_{12,108}$=3.86; p<0.01). Post hoc analysis showed a selective increase of DA in the shell. (figure 19.D). The correlation between percent of DA levels and nose poking is not significant in rats implanted in the NAc shell (r=-053; slope: p=0.28 NS) and in rats implanted in the NAc core (r=-0.14; slope: p=0.28 NS)

8th session: Two-way ANOVA showed an effect of area ($F_{1,9}$=27.33; p<0.01), of time ($F_{12,108}$=20.77; p<0.01) and an interaction area x time ($F_{12,108}$=6.87; p<0.01). Post hoc analysis showed a selective increase of DA in the shell (figure 19.E).

As shown in figure 23, a significant correlation between percent of DA levels and nose poking with r=0.43 and a significant slope (p<0.01) was obtained in the NAc shell, but not in the NAc core (r=0.12; slope: p=0,364NS).

The two slopes are statistically different ($F_{1,128}$=5.61, p=0.019).

10th session: Two-way ANOVA showed an effect of area (F_{17}=17.47; p<0.01), of time ($F_{12,84}$=13.21; p<0.01) and an interaction area x time ($F_{12,84}$=11.21; p<0.01). Post hoc analysis showed a selective increase of DA in the shell (figure 13.F).

As shown in figure 24, a significant correlation between percent of DA levels and nose poking with r=0.33 and a significant slope (p<0.01) was obtained in the NAc shell, but not in the NAc core (r=0.17; slope: p=0.25 N.S.). The two slopes are not statistically different ($F_{1,104}$=2.399, p=0.1244).

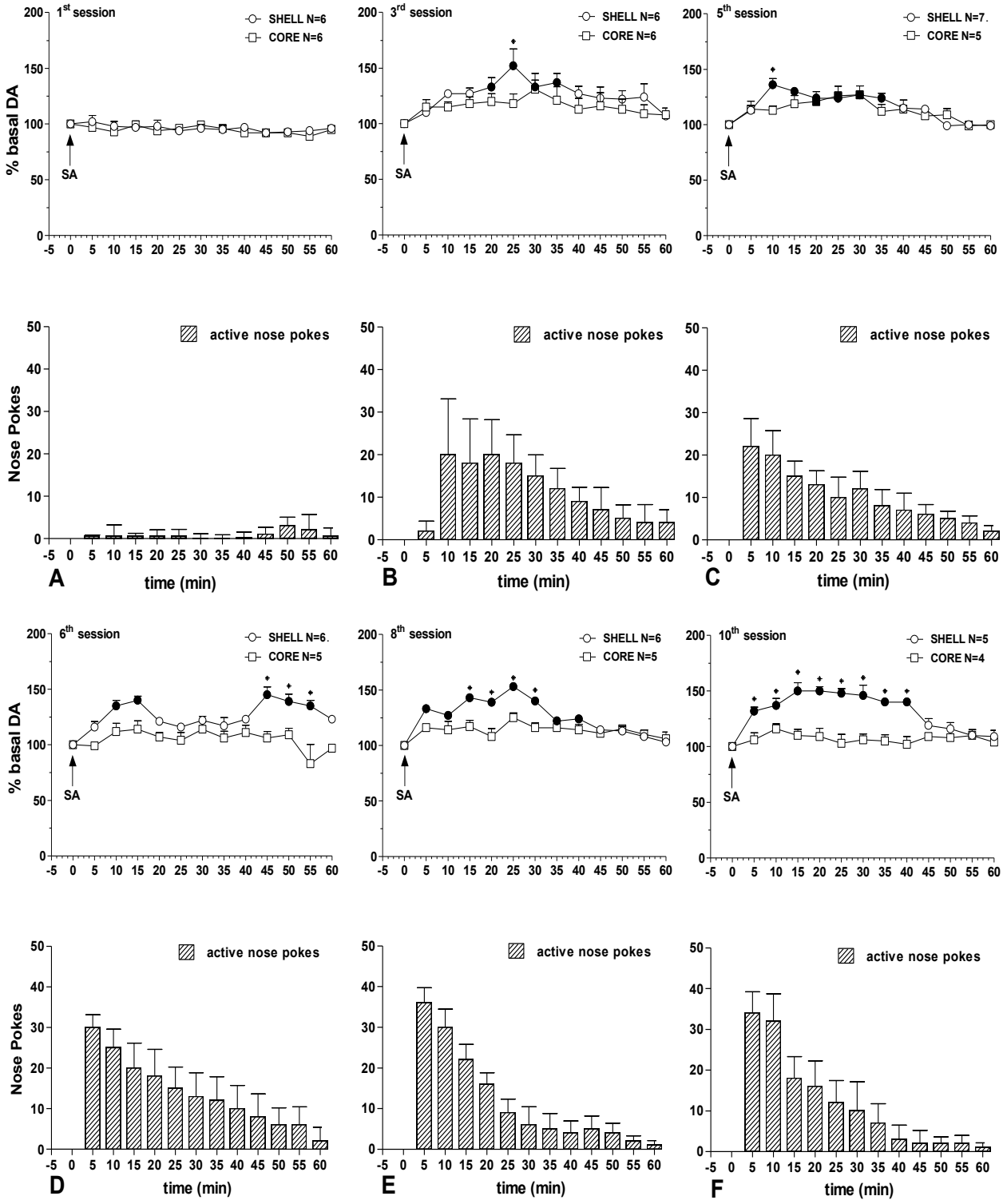


Figure 19: Evolution of the time-course of dialysate DA in the NAc shell (circles) and core (squares) and of active nose pokes (bars, means of shell and core group) on successive sessions during training on FR1 responding for sucrose.

Basal values of DA (means±SEM) in 5-min samples were as follow: NAc shell 26±3 fmoles (N=36), core 25±4 fmoles (N=31). Data are means±SEM. of the results obtained in the number of rats indicated in the figure.

Filled symbols: $p<0.05$ vs basal values;

*: $p<0.05$ vs values obtained in the core.

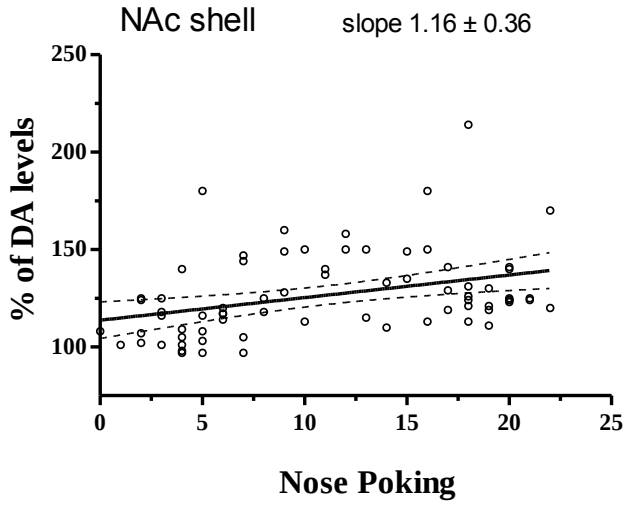


Figure 21: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose on the 3rd session.
Graph shows the correlation between of DA output in the NAc shell (N=6) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

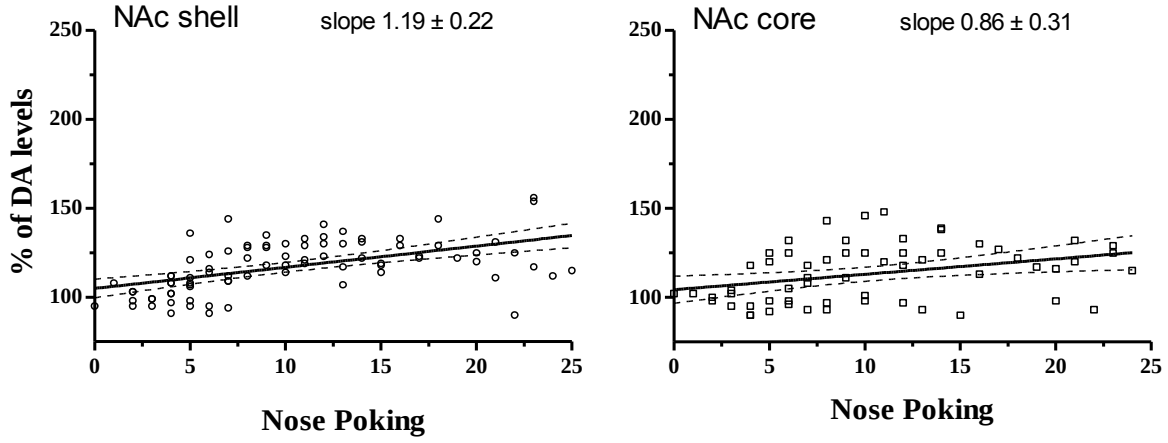


Figure 22: Regression analysis of the relationship between DA levels in the NAc shell and core and nose poking activity during FR1 responding for sucrose on the 5th session
Graphs show the correlation between the DA output in the NAc shell (circles, N=7) and core (squares, N=5) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session

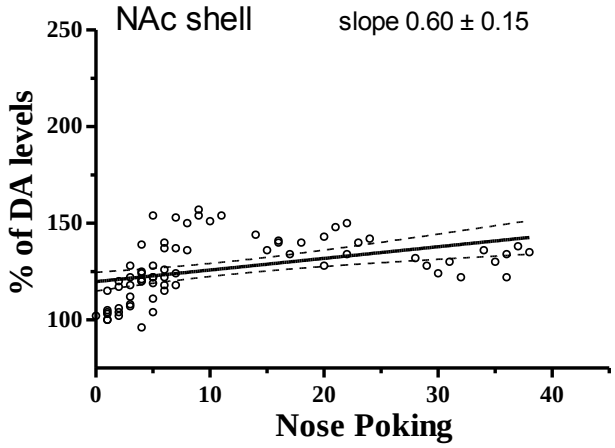


Figure 23: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose on the 8th session.
Graph shows the correlation between of DA output in the NAc shell (N=6) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

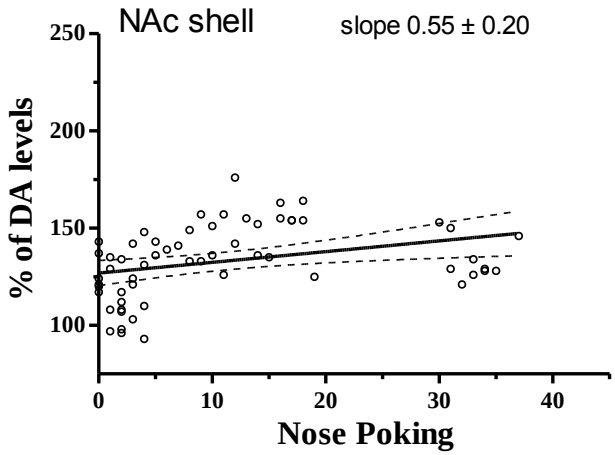


Figure 24: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose on the 8th session.
Graph shows the correlation between of DA output in the NAc shell (N=5) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

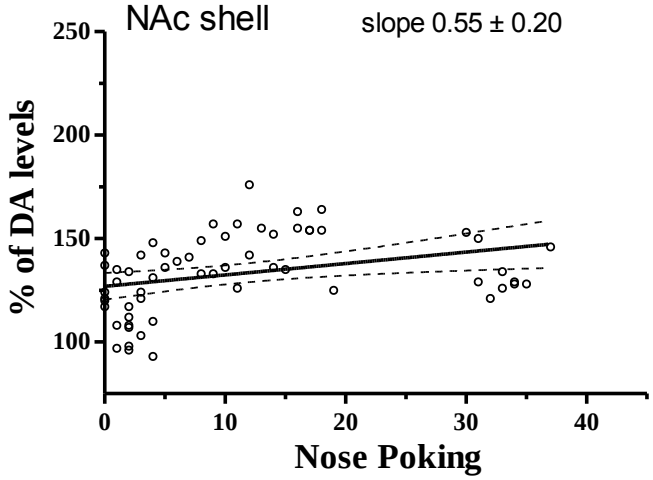


Figure 25: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose on the 10th session.
Graph shows the correlation between of DA output in the NAc shell (N=5) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

4.3.3 Responding for sucrose

Figure 26 (A) shows the time-course of DA in the NAc shell and core during FR1 responding for sucrose in rats that had been monitored with microdialysis during training.
Two-way ANOVA showed an effect of area ($F_{1,7}$=27.15; p<0.01), time ($F_{12,84}$=8.32; p<0.01) and an interaction area x time ($F_{12,84}$=5.19; p<=0.01). Tukey's test showed an increase of DA in the shell.

As shown in figure 27, a significant correlation between percent of DA levels and nose poking with r=0.61 and a significant slope (p<0.01) was obtained in the NAc shell, but not in the NAc core (r=0.01; slope: p=0,79 N.S.). The two slopes are statistically different ($F_{1,104}$=15.52, p<0.0001).

4.3.4 Responding under extinction

Figure 26 (B) shows the time-course of dialysate DA in the NAc shell and core under extinction of FR1 responding for sucrose, in the rats that had been monitored with microdialysis during training.

Two-way ANOVA showed an effect of area ($F_{1,6}$=37.85; p<0.01), time ($F_{12,72}$=10.02; p<0.01) and a significant interaction area x time ($F_{12,72}$=9.14; p<0.01).Tukey's test showed an increase of DA in the shell but not in the core.

As shown in Figure 28 a significant correlation between percent of DA levels and nose poking with r=0.45 and a significant slope ($p<0.01$) was obtained in the NAc shell, but not in the NAc core (r=0.12; slope: p=0.42 NS). The two slopes are statistically different ($F_{1,104}=12.05$, $p<0.0001$).

4.3.5 Response to non-contingent sucrose feeding

Figure 26 (C) shows the time-course of DA in the NAc shell and core during the sucrose pellets passive administration by the operator, in rats that performed the microdialysis experiment during the acquisition phase.

Two-way ANOVA showed an effect of time ($F_{12,48}=41.81$; $p<0.01$) and an interaction area x time ($F_{12,48}=2.69$; $p<0.01$). Tukey's test showed an increase of DA both in the shell and in the core.

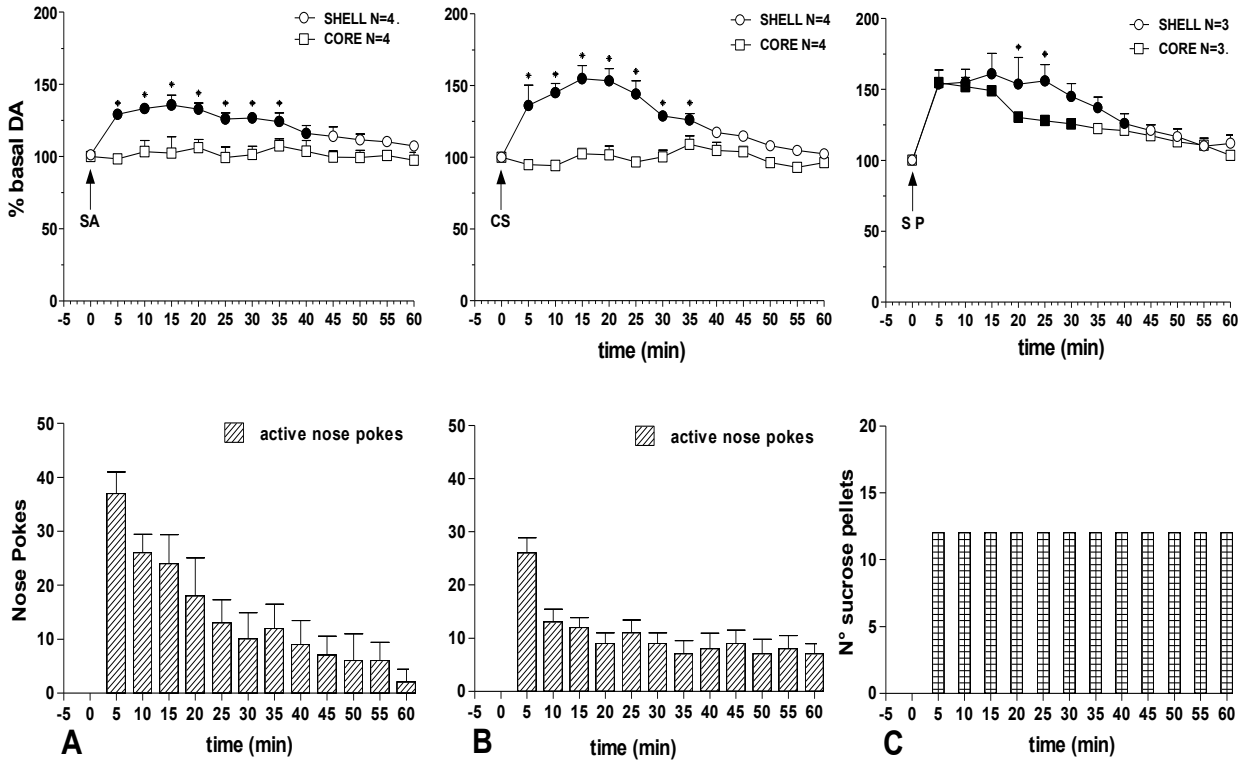


Figure 26: Time-course of DA in the NAc shell (circles) and core (squares) and active nose-pokes or number of pellets presented (bars) under FR1 responding for sucrose (A), extinction (B) and non-contingent sucrose pellet presentation in the rats monitored by microdialysis during training.

Basal values of DA (means±SEM) in 5-min samples were as follow: NAc shell 25±4 fmoles (N=11), core 24±3 fmoles (N=11).

Data are means±SEM. of the results obtained in the number of rats indicated in the figure.

Filled symbols: $p<0.05$ vs basal values;

*: $p<0.05$ vs values obtained in the core.

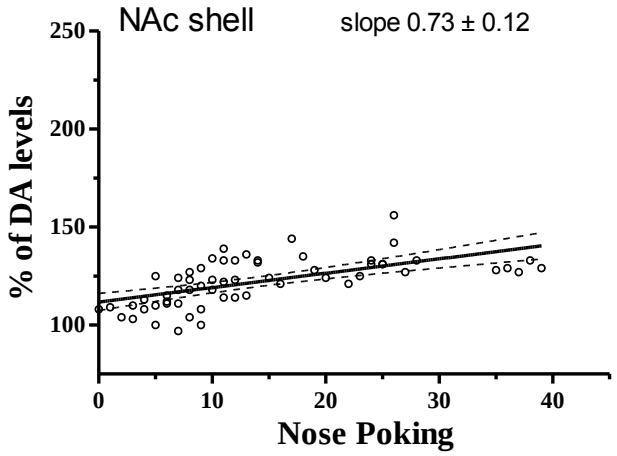


Figure 27: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during FR1 responding for sucrose.
Graph shows the correlation between of DA output in the NAc shell (N=5) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

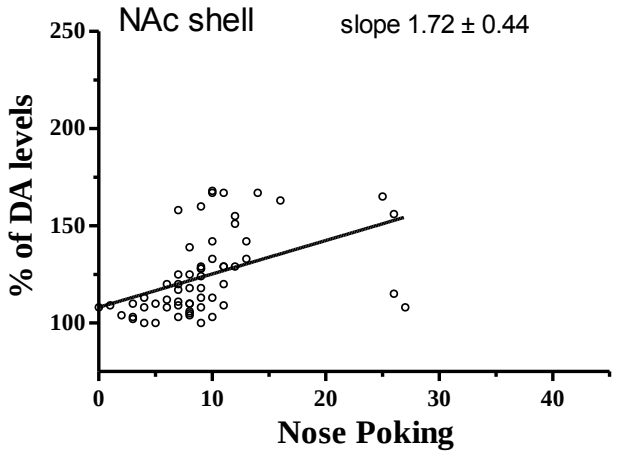


Figure 28: Regression analysis of the relationship between DA levels in the NAc shell and nose poking activity during the extinction session (FR1).
Graph shows the correlation between of DA output in the NAc shell (N=4) (Y-axis) and nose poking (X-axis) during sucrose pellets SA. Data are expressed as percent of DA levels during the 60-min period of microdialysis; nose poking is expressed as number of active nose pokes performed during the session.

5. Discussion

The main finding of the present study is that, in fully trained rats, FR1 and FR5 responding for sucrose activates DA transmission in the NAc shell and in the mPFCX but not in the NAc core. Extinction of FR1 responding in the presence of visual and auditory cues that signal sucrose availability was associated to a pattern of activation of DA transmission similar but shorter lasting than that of rats responding for sucrose. In contrast, in the same rats, feeding of sucrose pellets presented in a response non-contingent fashion was associated to activation of DA transmission also in the NAc core, in addition to the shell and mPFCX. In rats naïve to sucrose, NAc shell DA transmission was activated in response to feeding of sucrose presented non contingently and this effect underwent complete habituation. No habituation of DA response was observed upon sucrose feeding contingent upon FR1 and FR5 responding. Finally, within subjects monitoring of DA response in the NAc shell and core during training of FR1 responding for sucrose showed a progressive build-up of DA response in the NAc shell that was virtually maximal on the tenth trial with only transient and marginal activation of NAc core DA transmission on the 5thtrial.

In the two experimental groups dialysed at the end of training as well as in the group dialysed during training, active nose pokes increased progressively with training while inactive nose pokes remained quite low from the beginning, consistent with a strong dependency of responding from its outcome (see below). After two-three weeks (10-15 sessions) rats reached asymptotic responding indicative of full acquisition. The number of asymptotic active nose pokes emitted was not different between rats implanted in the NAc shell and in the NAc core nor between the group dialysed during and at the end of FR1 training.

During the extinction session, visual and auditory cues that signal session start and reward availability as well as auditory cues that follow active nose-pocking (feeder switch and pellet release) were still except that the pellet was prevented from falling into the dispenser. Under these conditions rats responses were still emitted on the active nose poke but were short lived, consistent with a tight dependence of responding from its outcome and resulting, according to Dickinson and Balleine (2002), from an instrumental incentive learning mechanism.

Dopamine transmission and responding for sucrose

During responding for sucrose, rats trained and tested on FR1 and FR5 showed a similar response pattern of changes in dialysate DA. DA rapidly increased in the NAc shell and mPFCX while remained at basal levels in the NAc core. Changes in NAc shell DA were time-locked to active nose-poking activity; thus, return of dialysate DA to basal coincided with downshift of responding for sucrose.

These observations contrast with those of Sokolowski et al (1999) who found that the increase of dialysate DA in the NAc shell and core during responding for food was prolonghed well over the period of active (operant) responding for food. It was during this phase and not, as in our case, during the operant phase that Sokolowski et al (1999) did obtain a larger increase of dialysate in the NAc shell compared to the core. Since in the present experiments the increase of DA in the shell over that in the core occurred from the beginning of responding for sucrose, it is possible that the shell versus core differences observed in the present study arise from a mechanism different from that operating in the case of Sokolowski et al. (1999) For example, while in our case they might result from differences in the activation of DA release, in the case of Sokolowski et al (1999) they might arise from differences in the disposition of DA after its release such as, for example, a reduction of DA reuptake, that slows the clearance of released DA from the extracellular space. Indeed, as situation similar to that observed by Sokolowski et al (1999) in the NAc was observed by us in the mPFCX, were the increase of DA went on for two or more 10 min samples when active responding for sucrose had already down to low levels. In our case the differential relationship of NAc shell and mPFCX DA with responding is consistent with differences in the clearance of released DA in the two areas (Garris and Whightman, 1994; Jones et al, 1996). It should be noted, however, that a straight comparison of the time relationships between changes in dialysate DA and responding between our study and that of Sokolowski et al (1999) is made difficult by the differences in time sampling of dialysate DA, 5 min in our study, 30 min in the Sokolowski et al (1999) study. Another possibly relevant difference is that Sokolowski et al (1999) utilized food rather than sucrose pellets.

As far as regards other microdialysis studies comparing changes in NAc shell and core DA transmission in rats responding for food pellets, their results are quite in contrast with the present one as in general they did observe increases in dialysate DA both in the shell and core without significant differences between the two NAc subdivisions.

Ostlund et al (2011) monitored dialysate DA in rat striatal subregions including the NAc shell and core during instrumental conditioning for food on a random-ratio schedule of reinforcement involving variation in the amount of effort needed to earn rewards across tests. Under these conditions dialysate DA increased during responding for food in both the shell and core and no differences between the two NAc subdivisions were observed in hungry and sated subjects and on three different sessions. The reason for the discrepancy between our results and those of Ostlund et al (2011) is unclear but might derive from the many differences in experimental conditions that included use of grain-based food instead of sucrose pellets, bar pressing instead of nose poking, random-ratio instead of fixed ratio schedules. Interestingly, however, when changes in

dialysate DA were correlated on individual subjects with response rate and number of rewards earned in sated and hungry rats, a positive correlation was observed in the NAc shell but not in the core; on the other hand, when individual changes of dialysate DA were correlated with changes in response ratio as an expression of effort, a negative correlation was obtained in the core. Thus, increase in DA release during instrumental conditioning was positively correlated to earning of reward in the NC shell and negatively correlated to response effort in the core. Although the overall changes observed in the NC shell and core by Outland et al (2011) do not agree with ours, the correlations of individual changes in dialysate DA in each NC subdivision with reward and effort might provide an explanation for the present observation that response-contingent sucrose feeding, that is likely to be associated to higher effort than non-contingent feeding, fails to increase dialysate DA in the NC core, while response non-contingent feeding increases dialysate DA also in the core (see below).

Another study whose results should be compared with ours is that by Cheng and Feenstra (2006) who reported that in an FR1 learning paradigm made up of two sessions with an interval of 2 hours between sessions. Dialysate DA rapidly increased during each session and to a similar extent in both NAc subdivisions and the only differences between shell and core were observed on the first session in the rats that learned to criterion, since they showed a higher NAc shell DA response compared to the rats that did not learn to criterion. In the present study, as learning progressed and rats increased responding for sucrose, dialysate DA progressively increased selectively in the NAc shell in all sessions, except for the 3rd session, where an increase in the core was also observed. Therefore, in the present study, a build up of DA response was observed in the NAc shell as learning progressed while the response in the core was transitory and aborted early in the learning process.

Segovia et al (2011) have studied changes in dialysate DA sampled every 15 min in the NAc shell and core during responding for food pellets (Bioserv) in different groups of rats previously trained on FR1 and on FR5 schedules. Quite in contrast with our observations, Segovia et al did not observe changes in dialysate DA in any NAc subdivision during FR1 responding for food in rats previously trained on the same schedule. In rats trained on FR5 and monitored during the same schedule, dialysate DA increased both in the shell and in the core but to a larger extent in the first subdivision. Therefore, this last set of observations partially agree with ours.

Only few voltammetric studies have directly compared shell versus core DA responses in rats self-administering food. Comparison of these studies with the present one is difficult due to the basic differences between microdialysis and voltammetry (see Introduction). A recent fast scan cyclic voltammetry study performed on rats trained to self-administer sucrose on a FR1 schedule

shows that presentation of the visual-auditory cue signaling reward availability elicits a phasic increase of extracellular DA in the NAc shell and core that fades within 2 seconds, when the lever is extended into the chamber and response is emitted to obtain the reward. This cue-related response is larger in the NAc shell than in the core and is followed by a second response that takes place immediately after lever extension and selectively in the NAc shell DA. This second component of the DA change is lower and slower and coincides with sucrose reward, extending in some rats over 10 sec after cue presentation (Cacciapaglia et al, 2012).

The observations of Cacciapaglia et al (2012) suggest that under responding for sucrose extracellular DA is released both in the NAc shell and in the core by cues signalling reward availability and only in the NAc shell by the reward itself, most likely, the sucrose taste. This conclusion is consistent with previous observations from the same group showing that appetitive (saccarin) and aversive (quinine) tastes increase and respectively decrease extracellular DA in the NAc shell but do not affect DA in the core (Roitman et al, 2008; Wheeler et al, 2011)

In order to translate these voltammetric observations into microdialysis terms one should consider that the voltammetric recordings refer to the time relationship of DA changes around each response without considering that DA released at each response is not immediately cleared from the extracellular compartment but adds on and raises basal DA levels. In contrast to voltammetry, microdialysis does estimate absolute levels of extracellular DA and therefore is able to take into account the increase of mean extracellular DA levels brought about by the contribution of those individual DA transients to overall extracellular DA. One should also consider that the activity of DA reuptake is about 3 times higher in the NAc shell than in the core and that DA transients on each trial are higher and more prolonged in the NAc shell than in the core and are likely to raise extracellular DA to a larger extent than in the core. Taking the above considerations into account, one would predict, starting from the observations of Cacciapaglia et al (2012), that the final contribution of the DA response to dialysate DA levels in the core would be lower or even absent as compared to the NAc shell. Therefore we conclude that the voltammetric observations of Cacciapaglia et al (2012) are consistent with ours and might even provide a clue to their explanation at the sub-second level.

Dopamine transmission and extinction of responding for sucrose

In order to investigate if cues signalling reward availability and preceding response emission as well as cues triggered by active nose-pokes are able induce changes in DA transmission during responding for sucrose and in order to distinguish them from the action of sucrose, rats were monitored under extinction conditions. In rats dialysed after FR1 training as well as in rats dialyzed during FR1 training, DA increased in the NAc shell and mPFCX. In rats trained on FR5 DA

increased both in the NAc shell and core. However, the increase in the NAc core was low and late, taking place on the 3rd sample. On this schedule no change in dialysate DA was observed in the mPFCX. We have confirmed this observation in an additional series of subjects (results not shown) but we have no explanation for it.

These results are in line with voltammetric studies showing that cues signalling sucrose availability phasically release DA in the NAc shell and core (Cacciapaglia et al 2012). A different pattern of shell versus core DA activation was obtained by in a pavlovian conditioning paradigm involving conditioning of a food smell with taste of a palatable food. In this paradigm, presentation of the CS increased dialysate DA in the NAc core rather than in the shell (Bassareo et al, in preparation). Thus, an opposite patterns of activation of DA transmission in shell versus core is obtained following exposure to CSs depending on their pavlovian or instrumental nature: DA transmission in the shell is potentiated by food conditioned stimuli only when they are conditioned by an operant associative learning.

Dopamine transmission and non-contingent sucrose feeding

One of the aims of the present study was to compare the effect of response-contingent and response non-contingent sucrose feeding on in vivo DA transmission in the NAc shell and core. In naïve rats repeatedly fed sucrose pellets presented non contingentlydialysate DA increased on the first trial and this response habituated on a second and third trial 24 h. apart from each other. These observations extend to sucrose pellets the observations of previous studies from our and others laboratory after various palatable foods (Bassareo and Di Chiara, 1997; 1999a e b, Gambarana et al., 2003; Rada et al., 2005; Danielli et al., 2009).

In rats previously trained to respond for sucrose on FR1 and FR5, response non–contingent feeding of sucrose pellets at the same mean rate at which the rats self-administer sucrose, elicited a robust and sustained increase in dialysate DA in the NAc shell and core and mPFCX. Since the same rats have been fed with sucrose during training and not earlier than two days before, this observation indicates that training to respond for sucrose eliminates habituation in the NAc shell.

This however does not mean that the release of DA induced by food is the effect of the primary stimulus properties (taste) of sucrose, as might be the case of naïve rats fed with sucrose. Sucrose is provided with taste as well as post-ingestive (e.g. metabolic) primary rewarding properties that can both act as primary stimuli. For example taste might lose its DA stimulant properties as a primary reward following repeated feeding but might gain conditioned DA stimulant properties by being predictively associated during operant training with sucrose post-ingestive rewarding properties. This might also explain the ability of non-contingent sucrose to stimulate DA transmission in the NAc core.

However, independently of the conditioned or unconditioned nature of the DA response to response non-contingent sucrose, the same sucrose stimulus differentially activates NAc core DA depending on the fact that, in the same subjects, it is fed contingently or non-contingently upon a response.

In order to interpret this observation, it is important to consider that a salty palatable food like Fonzies® also increases dialysate DA both in the NAc shell and core (Bassareo and Di Chiara, 1997). In view of this the NAc DA response to non-contingent sucrose would be regarded as qualitatively similar to that observed in untrained rats. A closer examination of the DA time-course, however, reveals that the response of NAc shell DA to non-contingent feeding is slower than that of response contingent, both on FR1 and FR2. While on FR1 the time-course of DA after non-contingent feeding is superimposable to that in the core, on FR5 the response in the core is slower than in the shell. It is possible therefore that, as a result of operant training, the NAc shell DA response to non-contingent sucrose, like that to contingent sucrose, is conditioned in nature, being related to the predictive association of intrinsic sucrose stimulus properties (e.g. smell, taste) with its post-ingestive (e.g.caloric) properties. The possibility that the DA stimulant properties of sucrose are unconditioned in untrained rats but become conditioned in rats trained to self-administer sucrose, provides in turn an interesting explanation of the lack of habituation of DA transmission in the NAc shell in rats trained to respond for sucrose.

As to the ability of non-contingent sucrose to stimulate DA in the NAc core, the fact that is not observed under responding for sucrose suggests that it is an unconditioned effect of food and that when sucrose is earned contingently upon a response its ability to stimulate DA transmission in the NAc core is actively suppressed. We speculate that suppression of activation of DA transmission in the core would prevent automatic, inappropriate, eventually species specific responses that would otherwise interfere with focusing responding on earning sucrose.

Dopamine transmission during training

An important observation made during the course of the present study is that in rats trained to respond for sucrose, while contingent sucrose feeding activates DA only in the NAc shell, non contingent sucrose feeding activates DA transmission both in the NAc shell and core. If indeed, as we suggested, contingent sucrose feeding inhibits the ability of sucrose feeding to stimulate DA transmission in the core, one would expect that this change would take place during training but would disappear as training is completed. In order to test this hypothesis, rats were monitored during training of FR1 responding for sucrose.

It is notable that on the 5^{th} trial, dialysate DA increased significantly not only in the shell but also in the core. However, from the 6^{th} trial on, the increase was limited to the NAc shell. On the

final trials, once training had been completed, DA increased only in the NAc shell under responding for sucrose and under extinction while increased both in the shell and in the core when sucrose was presented and fed non contingently.

Therefore, stimulation of DA transmission in the core takes place at the beginning of training and is lost as training is completed, consistently with the hypothesis that the response in the core is an unconditioned effect of sucrose that is inhibited when sucrose is obtained contingently.

Conclusions

The present study shows that under operant conditions, responding for sucrose stimulates DA transmission in the NAc shell and mPFCX but not in the core. As non contingent sucrose presentation and feeding activates DA also in the NAc core, it is hypothesized that operant responding for sucrose inhibits the ability of sucrose to stimulate DA transmission in the core. This inhibition might serve to inhibit impulsive and inappropriate responses, thus increasing the efficiency of goal-directed action.

No habituation of NAc shell responsiveness was obtained under operant sucrose feeding. This observation, coupled to the fact that responding under extinction was associated to stimulation of NAc shell but not of core DA suggests that activation of DA transmission during responding for sucrose is the effect of discriminative/conditioned stimuli and not of the unconditioned stimulus properties of sucrose. This might also apply to the stimulation of DA transmission in the NAc shell by non-contingent sucrose presentation and feeding.

This study provides a robust and reproducible model for a parametric study the relationship between behaviour and DA transmission in the NAc shell and core and in the mPFCX.

6. References

Alexander, G.E., & Crutcher, M.D. (1990). Functional architecture f basal ganglia circuits: neural substrates of parallel processing. Trends in Neurosciences , 13: 266-271

Alheid GF, Heimer, L (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience. Oct;27(1):1-39. Review.

Alheid, G.E., Heimer, L., Switzer, R.C. (1990). Basal Ganglia. In: The Human Nervous System. Paxinos (Ed), San Diego: Academic Press.

Anselme, P. (2009). The effect of exposure to drugs on the processing of natural rewards. Neuroscience & Biobehavioral Reviews , 33 : 314-335.

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. Journal of Neuroscience 17, 851–861.

Bassareo, V. and Di Chiara, G. (1999). Differential responsiveness of DA trasmission to food-stimuli in nucleus accumbens shell/core compartments. Neuroscience 89, 637-641 (a).

Bassareo, V. e Di Chiara, G. (1999). Modulation of feeling-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. European Journal of Neuroscience 11, 4389-4397 (b).

Bassareo V, De Luca MA, Di Chiara G (2002). Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. J Neurosci. Jun 1;22(11):4709-19.

Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G. (2003). Differential adaptive properties of accumbens shell dopamine responses to ethanol as a drug and as a motivational stimulus. Eur J Neurosci. 2003 Apr;17(7):1465-72.

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 (Berl). 2007 Apr;191(3):689-703. Epub 2006 Oct 28.

Berridge, K.C., & Robinson, T.E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research – Brain Research Reviews, 28: 309-369

Bindra D. (1974). A motivational view of learning, performance, and behavior modification. Psychol Rev. 1974 May;81(3):199-213.

Blanc et al., 1980; Blanc et al., 1980; Blanc, G., Herve, D., Simon, H., Lisoprawski, A., Glowinski, J., & Tassin, J.P. (1980). Response to stress of mesocortical-frontal dopaminergic neurons after long-term isolation. Nature, 284: 275-276.

Brauer, K., Häuber, M., Härtig, W., & Arendt, T. (2000). The core-shell dichotomy of nucleus accumbens in the rhesus monkey as revealed by double-immunofluorescence and morphology of cholinergic interneurons. Brain Research 858: 151-162.

Brown HD, McCutcheon JE, Cone JJ, Ragozzino ME, Roitman MF. (2011). Primary food reward and reward-predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum. Eur J NeurosciDec;34(12):1997-2006. doi: 10.1111/j.1460-9568.2011.07914.x. Epub Nov 29.

Cacciapaglia F, Saddoris MP, Wightman RM, Carelli RM (2012). Differential dopamine release dynamics in the nucleus accumbens core and shell track distinct aspects of goal-directed behavior for sucrose. Neuropharmacology. 2012 Apr;62(5-6):2050-6. doi: 10.1016/j.neuropharm.2011.12.027. Epub Jan 12.

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. Neuroscience. 1999 May;90(2):447-55.

Chang L, Haning W. .(2006). Insights from recent positron emission tomographic studies of drug abuse and dependence. Curr Opin PsychiatryMay;19(3):246-52. Review.

Cheng J, Feenstra MG. (2006). Individual differences in dopamine efflux in nucleus accumbens shell and core during instrumental learning. Learn Mem. 2006 Mar-Apr;13(2):168-77.

Cuadra G, Zurita A, Lacerra C, Molina V. (1999). Chronic stress sensitizes frontal cortex dopamine release in response to a subsequent novel stressor: reversal by naloxone. Brain Res Bull. 1999 Feb;48(3):303-8.

Curtis CE, D'Esposito M. (2003) Persistent activity in the prefrontal cortex during working memory.Trends Cogn Sci. 2003 Sep;7(9):415-423.

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. 2010 Jan;112(2):531-41. doi: 10.1111/j.1471-4159.2009.06483.x. Epub 2009 Nov 6.

Deutch et al., 1985; Deutch et al., 1985; Deutch, A.Y., & Cameron, D.S. (1992). Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience, 46: 49-56.

Di Chiara, G., (1990). In vivo brain dialysis of neurotransmitters. Trends in Pharmacological Sciences , 11: 116-11(3):116-121.

Di Chiara G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res. 2002 Dec 2;137(1-2):75-114. Review.

Di Chiara, G., & Bassareo, V. (2007). Reward system and addiction: what dopamine does and *doesn't do.* Current Opinion in Pharmacology , 7: 69-76.

Balleine BW, Dickinson (1998). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. A. Neuropharmacology. Apr-May;37(4-5):407-19. Review.

Drevets WC, Gautier C, Price JC, Kupfer DJ, Kinahan PE, Grace AA, Price JL, Mathis CA. (2001). Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol PsychiatryJan 15;49(2):81-96.

Feenstra MG, Botterblom MH.(1996). Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res. Dec 2;742(1-2):17-24.

Fenu S, Bassareo V, Di Chiara G. (2001). A role for dopamine D1 receptors of the nucleus accumbens shell in conditioned taste aversion learning. J Neurosci. Sep 1;21(17):6897-904.

Fenu and Di Chiara, (2003). Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D1 receptors. Eur J Neurosci. Oct;18(7):2025-30.

Feenstra MG, Botterblom MH.(1996) Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res. 1996 Dec 2;742(1-2):17-24.

Gambarana C, Masi F, 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(1):179-87.

Garris PA, Wightman RM. (1994). Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study. J Neurosci 14(1):442-50.

Gonon F, (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo.J Neurosci. 1;17(15):5972-8.

Grace, A.A. (2000). The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction, 95:S119-S128.

Groenewegen, H.J., & Russchen, F.T. (1984). Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. The Journal of Comparative Neurology , 223: 347-367.

HajnalA, Smith GP, Norgren R (2004) Oral sucrose stimulation increases accumbens dopamine in the rat.Am J Physiol Regul Integr Comp Physiol Jan;286(1):R31-7. Epub 2003 Aug 21.

Heimer L, Wilson RD. (1975) The subcortical projections of allocortex: similarities in the neuronal associations of the hippocampus, the piriform cortex and the neocortex. In:Santini M, editor. Golgi centennial symposium proceedings.New York: Raven Press, 1975:173_/93.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience41(1):89-125.

Heimer L., Alheid G.F., De Olmos J.S., Groenewegwn H.J., Haber S.N., Harlan R.E., Zahm D.S., (1997). The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci 9(3):354-381.

Hernandez L, Stanley BG, Hoebel BG (1986). A small, removable microdialysis probe. Life Sci. Dec 29;39(26):2629-37.

Jedema and Moghaddam, 1994; Jedema HP, Moghaddam B. (1994). Glutamatergic control of dopamine release during stress in the rat prefrontal cortex. J Neurochem. 1994 Aug;63(2):785-8.

JonesSR, O'Dell SJ, Marshall JF, Wightman RM. (1996). Functional and anatomical evidence for different dopamine dynamics in the core and shell of the nucleus accumbens in slices of rat brain. Synapse. Jul;23(3):224-31.

Jongen-Rêlo A.L., Voorn P., Groenewegen H.J. (1994) Immunohistochemical characterization of the shell and core territories of the nucleus accumbens in the rat. Eur J Neurosci. 6(8): 1255-64.

Kolb, B., & Whishaw, I.Q. (2003). Fundamentals of Human Neuropsychology (5 th Ed.). New York: Worth Publishers, Inc.

Lavin A, Nogueira L, Lapish CC, Wightman RM, Phillips PE, Seamans JK. (2005). Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. J NeurosciMay 18;25(20):5013-23.

Lecca D, Valentini V, Cacciapaglia F, Acquas E, Di Chiara G. (2006). Monitoring extracellular dopamine in the rat nucleus accumbens shell and core during acquisition and maintenance of intravenous WIN 55,212-2 self-administration. Psychopharmacology (Berl). Sep;188(1):63-74. Epub 2006 Jul 19.(b)

Lecca D, Valentini V, Cacciapaglia F, Acquas E, Di Chiara G. (2007). Reciprocal effects of response contingent and noncontingent intravenous heroin on in vivo nucleus accumbens shell versus core dopamine in the rat: a repeated sampling microdialysis study. Psychopharmacology (Berl). Sep;194(1):103-16. Epub 2007 Jun 2.(a)

Lecca D, Cacciapaglia F, Valentini V, Gronli J, Spiga S, Di Chiara G(2006). Preferential increase of extracellular dopamine in the rat nucleus accumbens shell as compared to that in the core during acquisition and maintenance of intravenous nicotine self-administration. Psychopharmacology (Berl). 2006 Mar;184(3-4):435-46. Epub Jan 6.(a)

Lecca D, Valentini V, Cacciapaglia F, Acquas E, Di Chiara G. (2007). Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat.Psychopharmacology (Berl). Apr;191(3):653-67. Epub 2006 Aug 24. (b)

Mogenson GJ, Jones DL, Yim CY. (1980). From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol. 1980;14(2-3):69-97. Review

Mogenson GJ, Yang CR. (1991). The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. Adv Exp Med Biol. 1991;295:267-90.

Ostlund SB, Wassum KM, Murphy NP, Balleine BW, Maidment NT. (2011) Extracellular dopamine levels in striatal subregions track shifts in motivation and response cost during instrumental conditioning. J Neurosci. Jan 5;31(1):200-7. doi: 10.1523/JNEUROSCI.4759-10.2011.

Paxinos, G., Watson, C., (1998). The rat brain in stereotaxic coordinates. 4th ed. Academic, New York.

Pinel, J.P.J. (2000). Biopsychology (4 th ed.). Boston: Allyn & Bacon.

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 U S A. 1995 Dec 19;92(26):12304-8.

Pontieri et al. 1996 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. 1996 Jul 18;382(6588):255-7.

Rada P, Avena NM, Hoebel BG.(2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience. 134(3):737-44.

Ramnani N., Owen AM. (2004). Anterior prefrontal cortex: insights into function from anatomy and neuroimaging. Nat Rev Neurosci. 2004 Mar;5(3):184-94.

Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM. (2004). Dopamine operates as a subsecond modulator of food seeking. J Neurosci. Feb 11;24(6):1265-71.

Roitman MF, Wheeler RA, Wightman RM, Carelli RM. (2008). Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci. Dec;11(12):1376-7. doi: 10.1038/nn.2219. Epub 2008 Nov 2.

Salamone JD. (1992). Complex motor and sensorimotor functions of striatal and accumbens dopamine: involvement in instrumental behavior processes. Psychopharmacology (Berl). 107(2-3):160-74. Review.

Sesack SR, Carr DB, Omelchenko N, Pinto A. (2003) Anatomical substrates for glutamate-dopamine interaction:evidences for specificity of connections and extrasynaptic actions. Ann N Y Acad Sci, 1003:36-52

Schultz W. (2002). Getting formal with dopamine and reward. Neuron 10;36(2):241-63. Review.

Segovia KN, Correa M, Salamone JD.(2011). Slow phasic changes in nucleus accumbens dopamine release during fixed ratio acquisition: a microdialysis study. Neuroscience. 2011 Nov 24;196:178-88. doi: 10.1016/j.neuroscience.07.078. Epub 2011 Aug 25.

Smith, D.A., & Bolam, P.J. (1990). The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. Trends in Neurosciences , 13: 259-266.

Sokolowski JD, Conlan AN, Salamone JD.(1998). A microdialysis study of nucleus accumbens core and shell dopamine during operant responding in the rat. NeuroscienceOct;86(3):1001-9.

Stolerman, I.P. (1992). Drugs of abuse: behavioural principles, methods and terms. Trends Pharmacol. Sci. 13, 170-176.

Surmeier, 2007 Surmeier, D.J. (2007). Dopamine and working memory mechanisms in prefrontal cortex. The Journal of Physiology, 581: 885.

Tanda G, Pontieri FE, Frau R, Di Chiara G. (1997). Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. Eur J Neurosci. Oct;9(10):2077-85.

Thierry, A.M., Tassin, J.P., Blanc, G., & Glowinski, J. (1976). Selective activation of the mesocortical DA system by stress. Nature , 263: 242-244.

Tzschentke, TM.(2001). Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog Neurobiol. Feb;63(3):241-320. Review.

Volkow ND, Wang GJ, Maynard L, Jayne M, Fowler JS, Zhu W, Logan J, Gatley SJ, Ding YS, Wong C, Pappas N. (2003). Brain dopamine is associated with eating behaviors in humans. Int J Eat DisordMar;33(2):136-42.

Voorn, P., Gerfen, C.R., Groenewengen, H.J. (1989). Compartmental organization of the ventral striatum of the rat: immunohistochemical distribution of enkephalin, substance P, dopamine, and calcium-binding protein. J Comp Neurol. 289(2):189-201.

Wheeler RA, Aragona BJ, Fuhrmann KA, Jones JL, Day JJ, Cacciapaglia F, Wightman RM, Carelli RM. (2011). Cocaine cues drive opposing context-dependent shifts in reward processing and emotional state. Biol Psychiatry. 2011 Jun 1;69(11):1067-74. doi: 10.1016/j.biopsych. 02.014. Epub Apr 8.

Wise RA. (1980). Action of drugs of abuse on brain reward systems. Pharmacol Biochem Behav;13 Suppl 1:213-23. Review.

Wise, R.A. (1987). The role of reward pathways in the development of drug dependence. Pharmacology & Therapeutics , 35: 227-263.

Zahm, D.S., & Brog, J.S. (1992). On the significance of subterritories in *the "accumbens" part of* the ventral striatum. Neuroscience , 50: 751-767.