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Dynamic interaction between medial prefrontal cortex and nucleus accumbens as a function of both motivational state and reinforcer magnitude: A c-Fos immunocytochemistry study

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Abstract

This study examined the effects of simultaneous variations in motivational state (food deprivation) and reinforcer magnitude (food presentation) on c-Fos immunoreactivity in the pre-and infralimbic medial prefrontal cortex (mPFC), nucleus accumbens (NAcc) core and shell, and dorsal striatum. In the first experiment, c-Fos was reliably increased in pre- and infralimbic mPFC of animals 12- and 36-h compared to 0-h deprived. In the second experiment, a small meal (2.5g) selectively increased c-Fos immunoreactivity in both mPFC subdivisions of 36-h deprived animals, as well as in both NAcc subdivisions of 12-h deprived animals. Correlational analyses revealed a changing relationship between mPFC subregions and the NAcc compartments to which they project. In subjects 12-h deprived and allowed a small meal, c-Fos counts in prelimbic mPFC and NAcc core were positively correlated, as were those in infralimbic mPFC and NAcc shell (r = . 83 and .76, respectively). The opposite was true of animals 36-h deprived, with prelimbic mPFC/NAcc core and infralimbic mPFC/NAcc shell negatively correlated (r = -.85 and -.82, respectively). The third experiment examined the effects of unrestricted feeding (presentation of 20g food) after 0, 12, or 36-h deprivation. No differences between mean c-Fos counts were found, though prelimbic mPFC/NAcc core, and mPFC/NAcc shell were positively correlated in animals 36-h deprived (r = .76 and .89, respectively). These data suggest that the activity within the mPFC and NAcc, as well as the interaction between the two, change as a complex combinatorial function of motivational state and reinforcer magnitude. Section: Cognitive and Behavioral Neuroscience

Keywords

food-deprivation; prefrontal cortex; nucleus accumbens; motivation; food reinforcement; rat

Introduction

A wide body of research implicates mesencephalic dopamine (DA) neurons in the processing of reinforcing stimuli (Satoh et al, 2003;Schultz, 2004,2006). Of particular interest are ventral tegmental area (VTA) DA cells, the firing rates of which change in response to the presentation of various reinforcers, including food (Ljungberg et al., 1992;Schultz, 1986). VTA DA neurons send a dense projection to the nucleus accumbens (NAcc) and thus provide a major source of synaptic input to that structure (Lindvall et al., 1974). Indeed, NAcc DA release, as well as

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changes in the firing of NAcc cells, occur in response to both food consumption and conditioned stimuli paired with food (Bassareo et al., 2002;Dalta et al., 2002;Roitman et al., 2005;Schultz et al., 1992). Inactivation of VTA diminishes the degree to which reinforcing stimuli can drive the activity of NAcc neurons while also attenuating the expression of reinforced behavior (Yun et al., 2004). This suggests a robust relationship between VTA, NAcc, and the manifestation of appetitive responses.

The VTA also sends a dopaminergic projection to the medial prefrontal cortex (mPFC; Lindvall et al., 1974). Neurochemical and electrophysiological analyses of mPFC reveal that this region responds to the presentation of many of the same stimuli that evoke changes in the subcortical regions mentioned above (Bassereo & Di Chiara, 1997; Bassareo et al., 2002; Richardson & Gratton, 1998). Indeed, a functional relationship, underpinned by a well described neuroanatomy, exists between mPFC, VTA and NAcc. Pyramidal cells in mPFC, which receive DA-ergic input from the VTA, project to NAcc, where they terminate on cells receiving DA input from the VTA. Furthermore, mPFC neurons feed-back onto the very VTA cells that project to mPFC and NAcc (for reviews see Sesack et al., 2003;Tzschentke, 2001). These connections allow mPFC to modulate activity in both NAcc and VTA, thus influencing the neuronal response of these regions to reinforcing stimuli, and consequently altering the behavioral response to such stimuli (Duvachelle et al., 1998;Jackson et al., 2001;Mitchell & Gratton, 1992;Thompson & Moss, 1995). Together, the above brain structures comprise a system critical for the processing of reinforcing events.

While a good deal of research focuses on the individual role of each of these regions (with regard to motivation and/or reinforcement), the interaction between these regions in the processing of such stimuli has received less attention. Furthermore, very little research has focused on the changing interaction between these regions across different motivational states of the organism. To this end, the current study describes three experiments that assay the neural consequences of varying the magnitude of both the motivational state (i.e. food deprivation) and the reinforcer presented (i.e. food). In all three studies, animals were food-deprived for 0, 12 or 36-h and then presented with either no food, a small meal (2.5g of sweetened 45mg pellets), or a large meal (20g of the same pellets). The neural consequences of these motivational (hunger) and reinforcing events (food presentation) were assessed. Via the use of c-Fos immunoreactivity, a marker of neuronal activation (Morgan & Curran, 1991; Sheng & Greenberg, 1990), the activity of five brain regions was simultaneously quantified; these were pre- and infralimbic mPFC, NAcc core and shell, and dorsal striatum. Further, it is worth noting that increased levels of ΔFosB, the protein product of another member of the *fos* family of genes, in the NAcc is associated with increased food motivation (Olausson et al., 2006;Cagniard et al., 2006).By generating neuroanatomical maps of c-Fos immunoreactivity, we were able to discern a fluid and co-varied pattern of activation across the component structures of the mesocorticolimbic DA system that changed as a function of both consumption and the animal's motivational state.

Results

Experiment 1: no meal

Separate one-way independent sample ANOVAs were computed on the c-Fos counts across deprivation levels for each brain region. Of the five brain regions examined, only two yielded statistically significant differences in c-Fos counts across the three food deprivation conditions (in pre- and infralimbic mPFC). Fig. 1, panels A and B depict mean (+ SEM) c-Fos counts in the pre/infralimbic prefrontal cortex and nucleus accumbens core/shell, respectively (though no significant results were found for NAcc in this experiment, differences between conditions were found for this region in the subsequent experiment). Analysis of the prelimbic prefrontal cortex revealed a significant main effect for group [$F(2,18)=3.883$, $p<.04$], and Tukey's HSD

post hoc comparisons confirmed a difference between 0-h and 12-h deprivation conditions ($p<.05$), as well as between 0-h and 36-h deprivation conditions ($p<.05$). The one-way ANOVA conducted on infralimbic prefrontal cortex revealed a similar main effect for group [$F(2,18)=9.950$, $p<.001$]. Post hoc tests yielded a significant difference between 0-h and 12-h groups ($p<.02$) and between 0-h and 36-h groups ($p<.01$).

A correlational analysis (Pearson's Product-Moment r-coefficient) was performed on Fos counts from prefrontal cortex and nucleus accumbens in all subjects. Specifically, on the basis of established anatomical connections (Berendse et al., 1992,Gabbott et al., 2005;Room et al., 1985), we examined whether prelimbic prefrontal cortex c-Fos counts were correlated with those of nucleus accumbens core, and whether infralimbic prefrontal cortex c-Fos counts were correlated with those of nucleus accumbens shell. No significant correlations were found.

Experiment 2: small meal (2.5g food)

As in Experiment 1, c-Fos counts were analyzed across the three groups (levels of food deprivation) for each of five distinct brain regions. One-way ANOVAs confirmed statistically reliable differences between groups on c-Fos counts in the prelimbic prefrontal cortex, infralimbic prefrontal cortex, nucleus accumbens shell, and nucleus accumbens core (Fig. 1, panels C and D). For the prelimbic prefrontal cortex [$F(2,18)=10.674$, $p<.01$], Tukey's HSD post hoc tests confirmed differences between 36-h and both 0-h and 12-h deprivation groups ($p<.05$). Similar results were obtained for the infralimbic prefrontal cortex [$F(2,18)=5.404$, $p<.04$], where post hoc tests also revealed a significant difference between 36-h and both 0-h and 12-h deprivation groups ($p<.05$). Finally, in the nucleus accumbens core [$F(2,18)=5.904$, $p<.05$], there were significant differences in c-Fos counts between 12-h and both 0-h and 36-h deprivation groups ($p<.05$); a similar trend was observed in the nucleus accumbens shell [$F(2,18)=4.812$, $p<.05$], with post hoc analyses revealing a difference between 12-h and both 0-h and 36-h deprivation conditions.

Further, we calculated the Pearson's r coefficient for c-Fos counts in prelimbic prefrontal cortex and nucleus accumbens core, as well as for infralimbic prefrontal cortex and nucleus accumbens shell; these data are represented in Fig. 2, panels D - F. These analyses revealed a significant positive correlation between accumbens core and prelimbic prefrontal cortex in animals 12h deprived (Fig 2, panel E; $n = 7$, $r = 0.834$, $p<.02$), as well as between accumbens shell and infralimbic prefrontal cortex in these same animals (Fig 2, panel E; $n = 7$, $r = 0.761$, $p<.04$). A similar correlational analysis of accumbens core and prelimbic prefrontal cortex in 36h subjects revealed a significant negative correlation (Fig 2, panel F; $n = 7$, $r = -0.852$, $p<.04$), as did an analysis of accumbens shell and infralimbic prefrontal cortex in those subjects (Fig2, panel f; $n = 7$, $r = -0.823$, $p< .02$).

Experiment 3: unrestricted feeding (20g)

Leftover food from the 20g applied to the individual enclosures was collected and weighed – this weight was subtracted from that of the total (20g) applied to each chamber to obtain amount consumed by each animal. One-way ANOVA revealed a significant effect for group [$F(2, 18)=20.985$, $p<.001$], and subsequent Tukey's HSD post hoc analyses confirmed a significant difference between animals 36-h deprived (mean $10.5 \pm .51$ g), and those 0 (mean 4.7 ± 1.0 g) and 12-h deprived (mean $5.7 \pm .92$ g; data not shown).

No significant differences were found in the c-Fos counts within any of the five brain regions that were examined ($p>.05$). As represented by Figure 2, panels G –I, correlational analyses revealed a strong relationship between c-Fos counts in the prelimbic prefrontal cortex and those in the nucleus accumbens core (Fig 2, panel I; $n=7$, $r = 0.764$, $p<.05$) of animals 12-h deprived,

as well as a similar correlation between the infralimbic prefrontal cortex and nucleus accumbens shell (Fig 2, panel I; n=7, r=0.891, p<.05) in the same animals.

Discussion

Changes in the motivational state of the animals (level of food deprivation) and/or presentation of a salient/reinforcing stimulus (food) produced profound alterations in the activity of cells (c-Fos counts) within the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAcc). Importantly, the nature of these alterations was highly dependent upon both the length of food deprivation and the magnitude of the reinforcer presented. The mPFC (this was true of both the pre- and infralimbic subregions) was most responsive to food deprivation, as neuronal activation in this region occurred when animals were food deprived and remained high in animals fed a small meal (2.5g) after 36-h deprivation. In contrast, the NAcc (again, this was true of both core and shell subregions) was most responsive to food reinforcement, but only if this reinforcement was of a low magnitude and given after 12 h of food deprivation. Finally, the activity of the mPFC and NAcc was significantly correlated, but only in animals that received a small meal after 12 or 36-h of food-deprivation, or a large meal after 36-h of food-deprivation. These results were restricted to mesocorticolimbic projection sites, as the dorsal striatum showed no effects across any of the motivational and/or reinforcment conditions in the three experiments.

As can be seen in Fig 1, the same relative pattern of c-Fos immunoreactivity was observed within each experiment for both the pre- and infralimbic subdivisions of mPFC -- as such, the current Discussion will address the region as a collective whole (i.e., the mPFC). As indicated above, food deprivation alone had an activating effect on mPFC, and c-Fos immunoreactivity in this region was elevated relative to non-deprived controls in animals both 12 and 36-h deprived. This result is consistent with the data of Carlson et al. (1987,1988), which indicate that 24 and 48-h of food deprivation serve to increase DA utilization in mPFC, but not NAcc (as discussed further below). A small meal of 2.5g food eliminated the difference between animals 12-h deprived and controls, whereas animals 36-h deprived required an average of 10.5g food to reduce mPFC activity to control levels. Thus, the activating effect of food deprivation on c-Fos immunoreactivity in mPFC was reversed by food consumption in a way that required animals to eat larger quantities of food after longer periods of deprivation. It therefore appears that in these studies mPFC c-Fos immunoreactivity was primarily or largely responsive to the motivational state of the organism.

This conclusion is particularly interesting when considered in the context of the putative functions of mPFC, in particular the formation of action-outcome associations (Baldwin et al., 2002;Corbitt & Balleine, 2003;Ostlund & Balleine, 2005). While learning can occur absent any motivational state (Tolman & Gleitman, 1949), it has long been observed that increasing motivation via deprivation facilitates the speed with which a food-reinforced instrumental response is acquired. If, indeed, mPFC is central to the process by which an organism associates some action of its own with the outcome of reinforcer delivery, then the current data (i.e., increased mPFC activity in hungry animals) suggest a neurobiological substrate by which the motivational state caused by hunger accelerates the process of operant learning. That is, the activation of mPFC by food deprivation might serve to facilitate the formation of the kinds of action-outcome associations necessary for the acquisition of instrumental behaviors.

As was the case for the mPFC, the current manipulations produced similar relative patterns of c-Fos immunoreactivity within the core and shell subregions of the NAcc. Therefore, once again, these two will be referred to collectively as NAcc. Neither food deprivation alone, nor feeding in an unrestricted fashion, produced differences in NAcc c-Fos immunoreactivity between deprived subjects and non-deprived controls. However, consumption of a small meal

selectively activated NAcc in animals 12-h deprived. These data are therefore consistent with the notion that the NAcc is engaged by the presentation of a reinforcing stimulus (Horvitz, 2002;Joseph et al., 2003). However, the same small meal did not increase activity in the 36-h deprived animals and motivational state had no differential effect in animals provided a large (20g) meal. That is, hunger alone was found to be insufficient to engage NAcc as was also shown by Carlson et al., (1987,1988) and the only condition in which NAcc activity rose above that of the non-deprived animals was with the presentation of a reinforcing stimulus (small meal) at 12-h of food deprivation. The lack of any reliable differences in NAcc c-Fos immunoreactivity across motivational states (Fig 1, Panel F) may be related to the fact that both food-deprived and non-deprived animals consumed some amount of food when allowed to eat in an unrestricted fashion (i.e., when given a large meal). As consumption of a palatable food has been shown to engage NAcc in non-deprived rats (Bassareo & Di Chiara, 1997), unrestricted feeding likely activated NAcc in non-deprived and deprived subjects alike. This suggests that consuming the amount appropriate for a given deprivation period may produce the same NAcc response independent of the subject's motivational state, even when the absolute amount of food ingested varies by deprivation level.

As revealed in Fig 2, mPFC and NAcc responded in concert to different combinations of reinforcer magnitude and motivational state. When animals were 12h deprived and allowed to consume a small amount of food, neuronal activation in these two DA terminal regions was positively correlated. Similarly, animals allowed unrestricted feeding after 36h of hunger responded with a strong positive correlation between mPFC and NAcc c-Fos immunoreactivity. A directionally opposite trend was observed in subjects allowed a small meal after 36h deprivation, and mPFC and NAcc activity were negatively correlated in these animals. Animals allowed *ad libitum* access to food prior to testing, animals allowed no food during testing, and animals allowed unrestricted feeding (i.e., given 20 g of food) subsequent to 12h deprivation showed no correlation between mPFC and NAcc activity. Thus, correlated neuronal activation in mPFC and NAcc occurred as a function of both reinforcer magnitude and motivational state. Specifically, both motivational state (which will engage the mPFC) and reinforcer presentation (which will engage the NAcc) are needed for any interaction between the mPFC and the NAcc to occur. Just as the processes of motivation and reinforcement are both separate yet inherently related, so too are the neuronal activation of mPFC and NAcc brain regions during deprivation and food presentation.

Converging lines of evidence suggest a functional relationship between mPFC and NAcc (Doherty & Gratton , 1996;Duvachelle et al., 1992;Duvachelle et al., 1998;Jackson et al., 2001;Mitchell & Gratton, 1992;Thompson & Moss, 1995). On the one hand, evidence suggests NAcc DA release can be inhibited by mPFC DA release, (Mitchell & Gratton, 1992) -- a relationship that could be mediated by mPFC projections to VTA that innervate very specific populations of VTA cells, including GABAergic interneurons that have an inhibitory effect on the mesoaccumbens DA projection (Carr & Sesack, 2000). Such reductions in DA levels in the NAcc can explain the decreased c-Fos activity in this area, as dopamine and dopamine agonists were shown to stimulate the induction of c-Fos in the nucleus accumbens and striatum (Cole et al., 1994;Graybiel et al., 1990;Moratalla et al., 1992;Nguyen et al., 1992). Thus, this interaction between DA efflux in mPFC and NAcc could account for the negative correlations between mPFC and NAcc Fos immunoreactivity observed in animals deprived for 36h and allowed to feed on a small meal. Also commensurate with these ideas are the data from animals 12h deprived that were allowed 2.5g of food. That is, a relative drop in mPFC DA in 12h subjects, as suggested by low c-Fos counts relative to 36h subjects, may have allowed for the robust NAcc response to the consumption of 2.5 g of food observed in these animals. Finally, though NAcc Fos counts were comparable in animals 0 and 36-h deprived and presented with a small meal, we argue that these effects, though similar, resulted from different responses to the presentation of food. For both groups the small meal represented a reinforcer that was very

low in magnitude. Animals 0-h deprived were not hungry prior to food presentation and their diminished motivational state may have served to devalue the reinforcer, resulting in lower NAcc activity in response to meal consumption. In contrast, animals 36-h deprived were too hungry to be satiated by such a small amount of food. This argument is also substantiated by high c-Fos counts in mPFC of 36-h deprived subjects, which contrasts with the low counts observed in mPFC of 12-h deprived animals after they consumed a small meal.

Another means by which the mPFC can modulate NAcc function involves a direct glutamatergic pathway projecting from the mPFC to the NAcc (Sesack et al., 2003;Tzschentke, 2001). For example, it has been demonstrated that electrical activation of cells within the mPFC produces an elevation in neuronal activity within the NAcc that cannot be easily accounted for by indirect activation via other brain structures (O'Donnell & Grace, 1995). This direct excitatory projection from mPFC to NAcc can account for the positive correlation observed between mPFC and NAcc c-Fos immunoreactivity seen in the 12-h/small meal group, as well as the correlation observed in animals 36-h deprived and allowed unrestricted feeding. In this situation, one would predict that higher activity in the mPFC should result in higher activity levels (and c-Fos immunoreactivity) in the NAcc – thereby rendering a strong positive correlation in the c-Fos counts of the two regions. Consistent with this idea are reports that glutamate can induce c-Fos in the striatum (Vanhoutte et al., 1999;Xia et al., 1996). Thus, it seems that under certain conditions the mPFC can inhibit the activity of the NAcc (possibly by inhibiting NAcc DA release), while under other conditions, and possibly via different cell populations, the mPFC can excite the NAcc. Such a formulation is consistent with a body of literature, cited above, suggesting a modulatory role for the mPFC in instrumental behavior.

We postulate that the observed correlations between the NAcc and mPFC are dependent on both the animal's motivational state and the magnitude of the reinforcer presented. When the motivational state is very high (e.g. 36-h food deprivation) a large reinforcer (e.g. presentation of 20g of food) produces a positive correlation; when the motivational state is moderate (e.g. 12-h food deprivation) a moderate reinforcer (e.g. 2.5g meal) will produce a positive correlation, as well. When, on the other hand, the motivational state is negligible (e.g. non-deprived animals or animals 12-h deprived and fed a large meal) no correlation occurs. Finally, when the motivational state is high and the reinforcer presented very small (e.g. animals 36-h deprived and fed 2.5g of food), a negative correlation is observed. Clearly, more research is needed to elucidate the nature of this covariation in mPFC and NAcc activity.

In summary, the current study was devised to examine the effects of a simultaneous manipulation of the motivational state of an animal (food deprivation), as well as the magnitude of the reinforcer presented that animal (food application), on the absolute and relative neuronal activity of the mPFC and NAcc. The results suggest that the mPFC is primarily responsive to level of food motivation – hunger alone increases mPFC activity, a small amount of food reverses this increase after a 12-h period of deprivation, while a larger meal was necessary to reverse the increase in animals with 36-h of deprivation. In contrast, the NAcc was unresponsive to changes in motivational state alone, and only exhibited an elevation in c-Fos activity in 12-h deprived animals given a small meal. Additionally, correlational analyses between c-Fos counts in the mPFC and NAcc of the same animals, provided evidence for a strong relationship between the two brain regions under varying motivational and reinforcement conditions. These data are consistent with other results already reported in the literature suggesting that mPFC can act to modulate neuronal activity within the NAcc. Moreover, these data suggest that the activity within each of these structures alone, as well as the interaction between the two, changes as a combinatorial function of the motivational state of the organism and the magnitude of reinforcer given to it.

Experimental Procedure

Subjects

The subjects were 75 male Sprague-Dawley rats obtained from Charles Rivers Laboratories (Hollister, CA). All subjects weighed between 310 and 350 grams at the beginning of each experiment. Upon receipt, the animals were gentled through daily handling on each of 5 days prior to the initiation of testing. The rats were individually housed in hanging plastic tubs located in the Psychology Department vivarium in a temperature controlled room (22°C) maintained on a 12:12 light-dark cycle (lights on at 0700h). Subjects had *ad libitum* access to food prior to food deprivation and *ad libitum* access to water throughout the experiment. All procedures were executed in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the UCSB Institutional Animal Care and Use Committee.

Experiment 1: no meal (deprivation only)

Testing occurred in 12 identical Plexiglas holding chambers (20 × 40cm). Subjects experienced a single 1 hour habituation session 48 hours prior to testing to permit acclimatization to the test chambers before the initiation of food deprivation. Subjects were then randomly assigned to one of three groups corresponding to three levels of food deprivation (the 0-h group, 12-h group and 36-h group; N = 7 rats/group). Food was removed from all 36-h animals at 2200h on the evening of the habituation session, and from all 12-h animals at the same time on the subsequent evening. The following day, subjects were taken from the home cage and placed into the Plexiglas chambers at 0900h after either 0, 11, or 35 hours of food deprivation, depending on the condition. After one hour in the enclosures the experimenter briefly opened the top of each chamber to simulate the administration of food in subsequent experiments – animals remained in the enclosures for an additional hour. Subjects were then promptly removed, anesthetized, and perfused (see *Perfusion* below).

Experiment 2: small meal (2.5g food)

Subjects were treated identically to those in Experiment 1 vis-à-vis habituation to the Plexiglas enclosures. They were then randomly assigned to one of three groups, each corresponding to a different duration of food deprivation (the 0-h, 12-h, or 36-h groups; N = 7 rats/group). Food was removed from the home cages for the designated deprivation periods and animals were placed in the Plexiglas chambers for testing on a timetable and in a manner identical to that used in Experiment 1. After one hour in the enclosures the experimenter administered a small meal consisting of 2.5g of 45mg sweetened pellets (Noyes). One hour later (post ingestion) animals were anesthetized and perfused. Any food left uneaten was removed from each enclosure and weighed.

Experiment 3: unrestricted feeding (20g food)

Subjects were treated identically to those in Experiment 1 and 2 vis-à-vis habituation to test chambers, random assignment to conditions, initiation of deprivation periods, and placement in the enclosures for testing. However, these rats were provided an opportunity to consume a large meal consisting of 20g of 45mg sweetened pellets in the test chambers after either 0, 12, or 36 hours of food deprivation. One hour after meal introduction, the rats were removed, anesthetized, and perfused. Food left uneaten was removed from each chamber and weighed.

Perfusion, tissue sectioning, and immunocytochemistry

As indicated above, one hour after subjects received either 0, 2.5, or 20g of food (Experiments 1, 2, or 3 respectively) animals were removed from the chambers, deeply anesthetized (intraperitoneal injection of .8-1 mL of sodium pentobarbital), and then transcardially perfused

with 60 mL of phosphate buffered saline (PBS) followed by 120 mL of 4% paraformaldehyde in PBS. The animals' brains were removed and placed in 20% sucrose in PBS. Using a cryostat (Leica CM 1800), brains were sliced into 40 m sections that were immediately mounted onto slides and then stored at -70°C for at least 24h prior to staining. A total of five brain regions were selected for analysis using the Paxinos and Watson (1998) atlas as a guide. The regions sampled from were mPFC, both pre- and infralimbic subdivisions (Bregma +3.20); dorsal striatum (Bregma +1.20); and NAcc, both core and shell subdivisions (Bregma +1.20), as described in prior work from our laboratory (Ben-Shahar et al.., 2004).

Brain samples from each of the three experiments were processed for c-Fos in three separate groups, one corresponding to each experiment. For immunocytochemistry, slides were washed in 0.05M Tris-buffered saline (TBS) between different treatments and stained using the avidin-biotin method using the Elite Vectastain Kit (Vector Laboratories, Burlingame, CA). Sections were treated with 0.25% Triton X-100 in TBS followed by 5% dimethyl sulfoxide (DMSO) in TBS and then, as a blocking step, incubated for one hour in a sealed humidity chamber in 20% normal goat serum (NGS) with 1% bovine serum albumin (BSA) and TBS. Slides were incubated for 24h in the primary antibody solution comprised of polyclonal rabbit anti-c-Fos (Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:1000, as well as 0.5% Triton X-100, 1% NGS, and TBS. Next, tissue samples were incubated for one hour in the secondary antibody mixture consisting of anti rabbit IgG, TBS and NGS, and then for thirty minutes in the avidin-biotinylated horseradish peroxidase complex. Staining was visualized using a solution of 0.05% of the chromogen diaminobenzidine (DAB), 0.01% H_20_2, and TBS. All slides were then dehydrated and coverslipped.

Cell counts

c-Fos immunoreactive cells were counted using at 40X magnification, using a microscope equipped with an eye-piece redicle that divided the visible field into a 10 × 10 grid (.25mm^2). Two trained raters, blind to group, counted from one of two adjacent samples for every target region. For the purposes of data analysis, the degree of c-Fos immunoreactivity within a given brain area was represented by the mean of the two raters' counts for that area.

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References

Baldwin AE, Sadeghian K, Kelley AE. Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. J Neurosci 2002;22:1063–71. [PubMed: 11826135]

Bassareo V, DeLuca MA, DiChiara G. Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. J Neurosci 2002;22:4709–19. [PubMed: 12040078]

Bassareo V, DiChiara G. Differential influence of associative and nonassociative mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed *ad libitum*. J Neurosci 1997;17:851–61. [PubMed: 8987806]

Ben-Shahar O, Ahmed SH, Koob GF, Ettenberg A. The transition from controlled to compulsive drug use is associated with a loss of sensitization. Brain Res 2004;995:46–54. [PubMed: 14644470]

Berendse HW, Galis-de Graaf Y, Groenewegen HJ. Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 1992;316:314–47. [PubMed: 1577988]

Cagniard B, Balsam PD, Brunner D, Zhuang X. Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward. Neuropsychopharm 2006;31:1362–70.

Carlson JN, Glick SD, Hinds PA, Baird JL. Food deprivation alters dopamine utilization in the rat prefrontal cortex and asymmetrically alters amphetamine-induced rotational behavior. Brain Res 1988;454:373–7. [PubMed: 3409020]

Carlson JN, Herrick KF, Baird JL, Glick SD. Selective enhancement of dopamine utilization in the prefrontal cortex by food deprivation. Brain Res 1987;400:200–3. [PubMed: 3815068]

Carr DB, Sesack SR. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with messoaccumbens and mesocortical neurons. J Neurosci 2000;20:3864–73. [PubMed: 10804226]

Cole DG, Kobierski LA, Konradi C, Hyman SE. 6-Hydroxydopamine lesions of rat substantia nigra up-regulate dopamine-induced phosphorylation of the cAMP-response element-binding protein in striatal neurons. Proc Natl Acad Sci U S A 1994;91:9631–35. [PubMed: 7937819]

Corbit LH, Balleine BW. The role of prelimbic cortex in instrumental conditioning. Behav Brain Res 2003;146:145–57. [PubMed: 14643467]

Dalta KP, Ahier RG, Young AMJ, Gray JA, Joseph MH. Conditioned appetitive stimulus increases dopamine in the nucleus accumbens of the rat. Euro J Neurosci 2002;16:1987–93.

Doherty MD, Gratton A. Medial prefrontal cortex D1 receptor modulation of the meso-accumbens response to stress: an electrochemical study in freely behaving rats. Brain Res 1996;715:86–97. [PubMed: 8739626]

Duvachelle CL, Fleming SM, Kornetsky C. Prefrontal cortex infusions of SCH 23390 cause immediate and delayed effects on ventral tegmental area stimulation reward. Brain Res 1998;811:57–62. [PubMed: 9804893]

Duvachelle CL, Levitin LA, MacConell M, Lee LK, Ettenberg A. Opposite effects of prefrontal cortex and nucleus accumbens infusions of flupenthixol on stimulant-induced locomotion and brain stimulation reward. Brain Res 1992;576:104–10. [PubMed: 1515903]

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 2005;492:145–77. [PubMed: 16196030]

Graybiel AM, Moratalla R, Robertson HA. Amphetamine and cocaine induce drug specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 1990;87:6912–6916. [PubMed: 2118661]

Horvitz JC. Dopamine gating of glutamatergic sensorimotor and incentive incentive motivational input signals to the striatum. Behav Brain Res 2002;137:65–74. [PubMed: 12445716]

Jackson ME, Frost AS, Moghaddam B. Stimulation of prefrontal cortex at physiologically relevant frequencies inhibits dopamine release in the nucleus accumbens. J Neurochem 2001;78:920–3. [PubMed: 11520912]

Joseph MH, Dalta K, Young AMJ. The interpretation of the measurement of nucleus accumbens dopamine by in vivo dialysis. Neurosci Biobehav Rev 2003;27:527–41. [PubMed: 14599434]

Konradi C, Leveque JC, Hyman SE. Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J Neurosci 1996;16:4231–4239. [PubMed: 8753884]

Lindvall O, Bjorklund A, Moore RY, Stenevi U. Mesencephalic dopamine neurons projecting to neocortex. Brain Res 1974;81:325–331. [PubMed: 4373129]

Ljungberg T, Apicella P, Schultz W. Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol 1992;67:145–63. [PubMed: 1552316]

Mitchell JB, Gratton A. Partial dopamine depletion of the prefrontal cortex leads to enhanced mesolimbic dopamine release elicited by repeated exposure to naturally reinforcing stimuli. J Neurosci 1992;12:3609–3618. [PubMed: 1527600]

Morgan JL, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. Ann Rev Neurosci 1991;14:421–51. [PubMed: 1903243]

Moratalla R, Robertson HA, Graybiel AM. Dynamic regulation of NGFI-A (zif 268, egr1) gene expression in the striatum. J Neurosci 1992;12:2609–2622. [PubMed: 1613551]

Nguyen TV, Kosofsky B, Birnbaum R, Cohen BM, Hyman SE. Differential expression of c-fos and zif 268 in rat striatum after haloperidol, clozapine and amphetamine. Proc Natl Acad Sci USA 1992;89:4270–4274. [PubMed: 1374894]

O'Donnell P, Grace AA. Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. J Neurosci 1995;84:3622–39.

Olausson P, Jentsch JD, Tronson N, Neve RL, Nestler EJ, Taylor JR. DeltaFosB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. J Neurosci 2006;26:9196–9204. [PubMed: 16957076]

Ostlund SB, Balleine BW. Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. J Neurosci 2005;25:7763–70. [PubMed: 16120777]

Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4. Academic Press; San Diego: 1998.

Richardson NR, Gratton A. Changes in medial prefrontal dopamine levels associated with response-contingent food reward: an electrochemical study in rat. J Neurosci 1996;18:9130–8. [PubMed: 9787015]

Roitman MF, Wheeler RA, Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 2005;45:587–97. [PubMed: 15721244]

Room P, Russchen FT, Goenewegen HJ, Lohman AHM. Efferent connections of the prelimbic (area 32) and the infralimbic (area 25) cortices: an anterograde tracing study in the cat. J Comp Neurol 1985;242:40–55. [PubMed: 4078047]

Satoh T, Nakai S, Sato T, Kimura M. Correlated coding of motivation and outcome of decision by dopamine neurons. J Neurosci 2003;23:9913–23. [PubMed: 14586021]

Sesack SR, Carr DB, Omelchenko N, Pinto A. Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. Ann NY Acad Sci 2003;1003:36–52. [PubMed: 14684434]

Schultz W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. J Neurophysiol 1986;56:1439–61. [PubMed: 3794777]

Schultz W. Neural coding of basic reward terms of animal learning theory, game theory, microeconomics and behavioural ecology. Curr Op Neurobiol 2004;14:139–47. [PubMed: 15082317]

Schultz W. Behavioral theories and the neurophysiology of reward. Ann Rev Psychol 2006;57

Schultz W, Apicella P, Scarnati E, Ljungberg T. Neuronal activity in monkey ventral striatum related to the expectation of reward. J Neurosci 1992;18:4595–610. [PubMed: 1464759]

Sheng M, Greenberg ME. The regulation and function of *c-fos* and other immediate early genes in the nervous system. Neuron 1992;4:477–85. [PubMed: 1969743]

Thompson TL, Moss RL. In vivo stimulated DA release in the nucleus accumbens: modulation by the prefrontal cortex. Brain Res 1995;686:93–8. [PubMed: 7583276]

Tolman EC, Gleitman H. Studies in spatial learning; place and response learning under different degrees of motivation. J Exp Psychol 1949;39:6539.

Tzschentke TM. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog Neurobiol 2001;63:241–320. [PubMed: 11115727]

Vanhoutte P, Barnier JV, Guibert B, Pages C, Besson MJ, Hipskind RA, Caboche J. Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. Mol Cell Biol 1999;19:136–146. [PubMed: 9858538]

Xia Z, Dudek H, Miranti CK, Greenberg ME. Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. J Neurosci 1996;16:5425–5436. [PubMed: 8757255]

Yun IA, Wakabayashi KT, Fields HL, Nicola SM. The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. J Neurosci 2004;24:2923–33. [PubMed: 15044531]

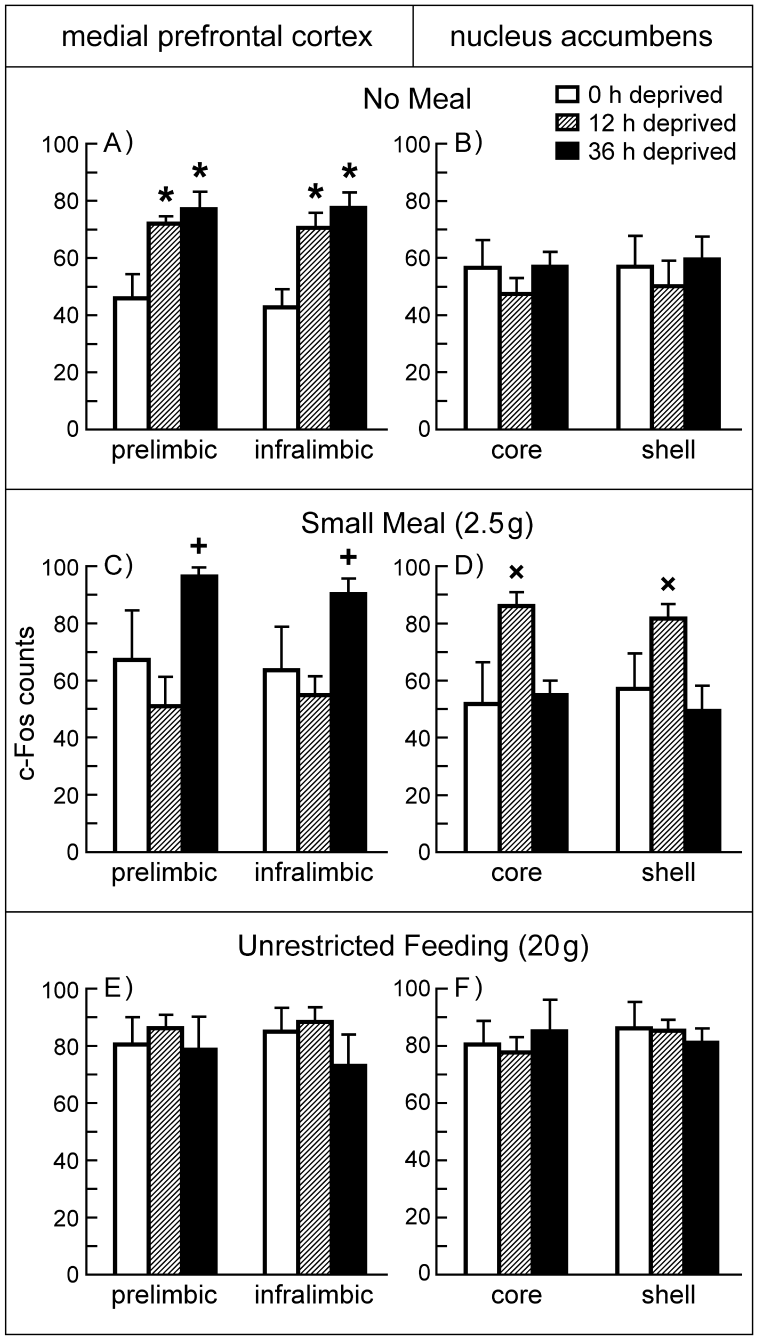

Fig 1.

Mean (+ SEM) c-Fos counts in response to variations in motivational state (either 0, 12, or 36-h food deprivation) and reinforcer magnitude (presentation of either 0, 2.5, or 20g food). *****Represents significant differences between both the 12 and 36-h deprived subjects and the non-deprived controls ($p<.05$). $^{+}$Represents significant differences between 36-h deprived subjects and both 12-h deprived subjects and non-deprived controls ($p<.05$). $^{\times}$Represents significant differences between animals 12-h deprived and both 36-h deprived subjects and non-deprived controls.

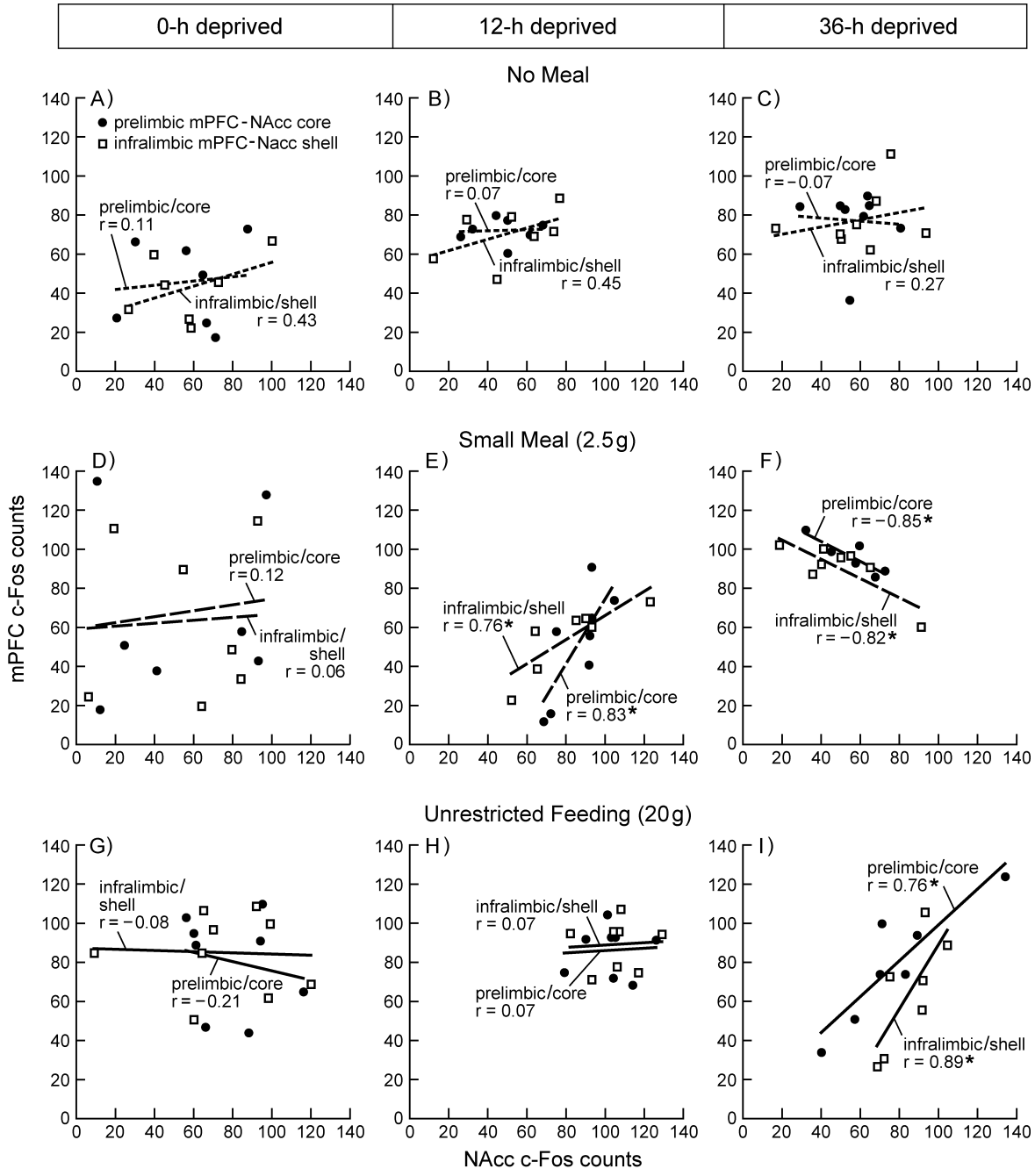

Fig 2.

Correlations between mPFC c-Fos counts (y-axis in all panels) and NAcc c-Fos counts (x-axis in all panels) in the same subjects. *Indicates a statistically significant correlation ($p<.05$).