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Molecular Brain Research 124 (2004) 134–142
MOLECULAR BRAIN RESEARCH
www.elsevier.com/locate/molbrainres

Research report

Opiate-like effects of sugar on gene expression in reward areas of the rat brain

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Accepted 26 February 2004

Available online 2 April 2004

Abstract

Drugs abused by humans are thought to activate areas in the ventral striatum of the brain that engage the organism in important adaptive behaviors, such as eating. In support of this, we report here that striatal regions of sugar-dependent rats show alterations in dopamine and opioid mRNA levels similar to morphine-dependent rats. Specifically, after a chronic schedule of intermittent bingeing on a sucrose solution, mRNA levels for the D2 dopamine receptor, and the preproenkephalin and preprotachykinin genes were decreased in dopamine-receptive regions of the forebrain, while D3 dopamine receptor mRNA was increased. While morphine affects gene expression across the entire dopamine-receptive striatum, significant differences were detected in the effects of sugar on the nucleus accumbens and adjacent caudate-putamen. The effects of sugar on mRNA levels were of greater magnitude in the nucleus accumbens than in the caudate-putamen. These areas also showed clear differences in the interactions among the genes, especially between D3R and the other genes. This was revealed by a novel multivariate analysis method that identified cooperative interactions among genes, specifically in the nucleus accumbens but not the caudate-putamen. Finally, a role for these cooperative interactions in a load-sharing response to perturbations caused by sugar was supported by the finding of a different pattern of correlations between the genes in the two striatal regions. These findings support a major role for the nucleus accumbens in mediating the effects of naturally rewarding substances and extend an animal model for studying the common substrates of drug addiction and eating disorders.

Theme: Neural basis of behavior
Topic: Drugs of abuse; Opioids and others

Keywords: Sucrose; Morphine; Nucleus accumbens; Caudate-putamen; Dopamine receptor; Preproenkephalin; Preprodynorphin; Preprotachykinin; Addiction; Reward

1. Introduction

The dopamine hypothesis of reward predicts that abused drugs and rewarding food substances activate some common pathways [32,69,70]. In support of this, drugs abused by humans and palatable food substances both elevate levels of extracellular dopamine in the striatal forebrain of the rat [19,31]. Moreover, gene expression alterations in the mesolimbic dopamine system, especially in the nucleus accumbens, are associated with drug addiction [45] and may also mediate some of the rewarding effects of saccharin and sucrose [29,41]. The effects of drugs of abuse on gene expression in ''reward'' regions of the rat brain have been well studied [26,33,49,60,74]. However, there is less known about the effects of food substances, such as sugar, on gene expression in these areas [10,13].

A major focus of expression studies in animal models of addiction has been on the opioid and dopamine systems. The enkephalin and dynorphin opioids, synthesized from the preproenkephalin (pE) and preprodynorphin (pD) genes, as well as the opioid-associated peptides from the preprotachykinin mRNA (pT), are abundant in forebrain

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doi:10.1016/j.molbrainres.2004.02.013

regions expressing the dopamine receptors, D1, D2 and D3. Considerable evidence for functional interactions between the opioid and dopamine systems has been collected during the past 20 years. Some evidence suggests, for example, that dopamine regulates expression of gene products of pE, pD and pT, and that these products act, directly or indirectly, in feedback loops that regulate dopamine tone [27,44,50,52,54,57,61,64].

Some recent evidence suggests that sugar dependency might involve alterations previously associated with the effects of addictive drugs. For example, when a sugar solution is made available to rats on a schedule that promotes intermittent bingeing, locomotor sensitization is observed in response to dopamine agonists [3], and there is increased susceptibility to withdrawal [13]. At the molecular level, similarities have been identified between the effect of morphine [26,58] and sucrose [10,14] in decreasing D2 dopamine receptor levels. In addition, a recent study shows that morphine and sucrose reward are both modulated by the transcription factor CREB [6]. These findings indicate that a more extensive similarity of effects of sugar and morphine might exist.

Recent developments in gene expression technologies have allowed measurement of alterations in large numbers of genes simultaneously [24,55]. Some methods for analyzing these alterations have used correlation analysis to identify subsets of genes, so that a smaller number of ''factors'' can be extracted. Most of these methods are based on strong model assumptions, such as a known functional relationship among the genes or with the underlying factors. One example of this is the application of Fourier analysis to the expression of genes through the cell cycle or the circadian rhythm [2,12,21,59]. While these approaches have been found useful in several applications, model-based results rely heavily on the validity of the assumptions underlying the model. For instance, combining several genes by means of a weighted sum of transformed univariate expression levels assumes that the relative importance of the genes and their covariance structure are known, and that the combinations are valid independent of the strength of the factor(s) underlying the differences measured. These assumptions, however, are unlikely to be justified in biological systems, especially as experience with targeted deletions of genes in mice has highlighted a system of interacting genes that can compensate for even the complete elimination of one member of the system, often leading to the absence of the expected phenotype in genetic mutants [9].

Recently, nonparametric methods based on u statistics, widely used for univariate [39] and interval-censored data [25], have been extended to the scoring of multivariate profiles [37,72,73]. With this approach, interactions among genes can be assessed without making assumptions regarding their correlation, providing a novel insight into interactions among genes in a heterogeneous tissue. Using this approach in the present study has identified interactions among genes, specifically in the nucleus accumbens, that fit a cooperative system of responses to perturbations, supporting the central role of the nucleus accumbens in mediating reward-related information.

2. Materials and methods

2.1. Samples

Male Sprague–Dawley rats were obtained from Taconic Farms (Germantown, NY) and housed individually in hanging wire cages on a reversed 12:12-h light/dark cycle. Experimental rats ($n=8$) were put on a 21-day schedule involving daily 12-h food deprivation followed by 12 h access to chow and 10% sucrose, starting 4 h into the dark period of the 12-h circadian cycle [13]. These rats show signs of opiate withdrawal when treated with naloxone, including teeth chattering, anxiety and distress vocalizations [14]. A control group ($n=8$) was treated similarly but without access to sucrose. Five hours into the dark cycle, all animals were sacrificed, and coronal slices were stabilized in RNALater (Ambion, Austin TX). Selected brain regions were later dissected, and RNA was extracted as described previously [58]. Tissue was teased out of coronal slices made in a brain matrix. The most anterior blade was placed at the olfactory tubercle, near the approximate location of the islands of Calleja. The next blade was placed 2 mm caudal to the first and the third blade was placed 2 mm further back. The nucleus accumbens forms a clearly defined structure after immersion in RNALater (50% ammonium sulfate), and it was removed from the ventral portion of the first coronal slice. On the second slice, the striatal tissue in the top 2/3 of the area beneath the cortex was teased out. This second slice also contains the back side of the ventral striatum as well as the globus pallidus, and these areas were avoided. With this dissection method, we believe there is minimal cross-contamination, with the nucleus accumbens containing consistently all the same subregions, and the dorsal striatum (caudate-putamen) containing striatal tissue. While there is the possibility that some small portion of ''nucleus accumbens'' tissue was included with the more posterior and dorsal ''caudate-putamen'' tissue, the opposite is less likely. Animals were handled following the guidelines of the NIH Guide for the Care and Use of Laboratory Animals.

2.2. qPCR

Tissue levels of mRNA were assayed by quantitative RT-PCR, as previously described [58]. The primer pairs for preproenkephalin (pE) (5′-AAA ATC TGG GAG ACC TGC AA-3′ and 5′-CAT GAA ACC GCC ATA CCT CT-3′) (GenBank #K02807.1) amplified a 242 base region of exon 3. The primer pairs for preprodynorphin (pD) (5′-GGG TTC GCT GGA TTC AAA TA-3′ and 5′-TGT GTG GAG AGG

GAC ACT CA-3′) (RefSeq #NM_019374) amplified 197 bases in the 3′ UTR of exon 4, approximately 150 bases before the polyadenylation site. The primer pairs for preprotachykinin (pT) (5′-AGC CTC AGC AGT TCT TTG GA-3′ and 5′-CGG ACA CAG ATG GAG ATG AA-3′) (RefSeq #NM_012666) amplified a region beginning 35 bases past the stop codon, flanking the alternative splice sites that generate the three known mRNA species: alpha (200 base amplicon), beta (254 base amplicon), and gamma (210 base amplicon) [38]. The primer pairs used in this study for D1, D2, D3, S-100 beta, SNAP-25, synaptophysin, beta actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the calcium modulated cyclophilin ligand (CaML) were described previously [58]. The D2 primers measured both isoforms of D2 [51]. The larger transcript was clearly the major band on end-point agarose gel analysis, but no differential quantification could be done at the end-point and no attempt was made to distinguish the

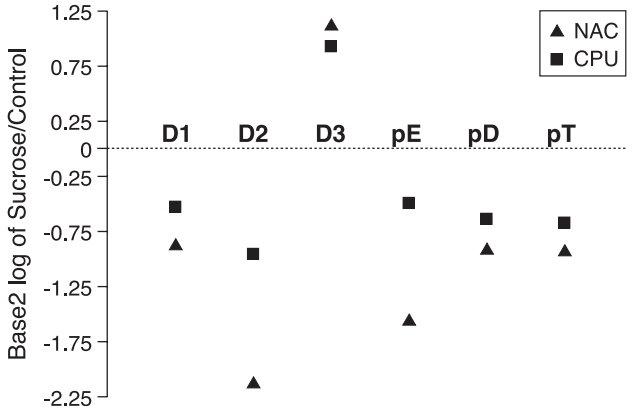

Fig. 1. Ratio of mean expression of sucrose-dependent to control rats for six genes in the nucleus accumbens (triangles) and the caudate-putamen (squares). The base 2 log of the ratio is shown. D1, D2, D3: dopamine receptors D1, D2 and D3; pE, preproenkephalin; pD, preprodynorphin; pT, preprotachykinin. $n = 6–8$.

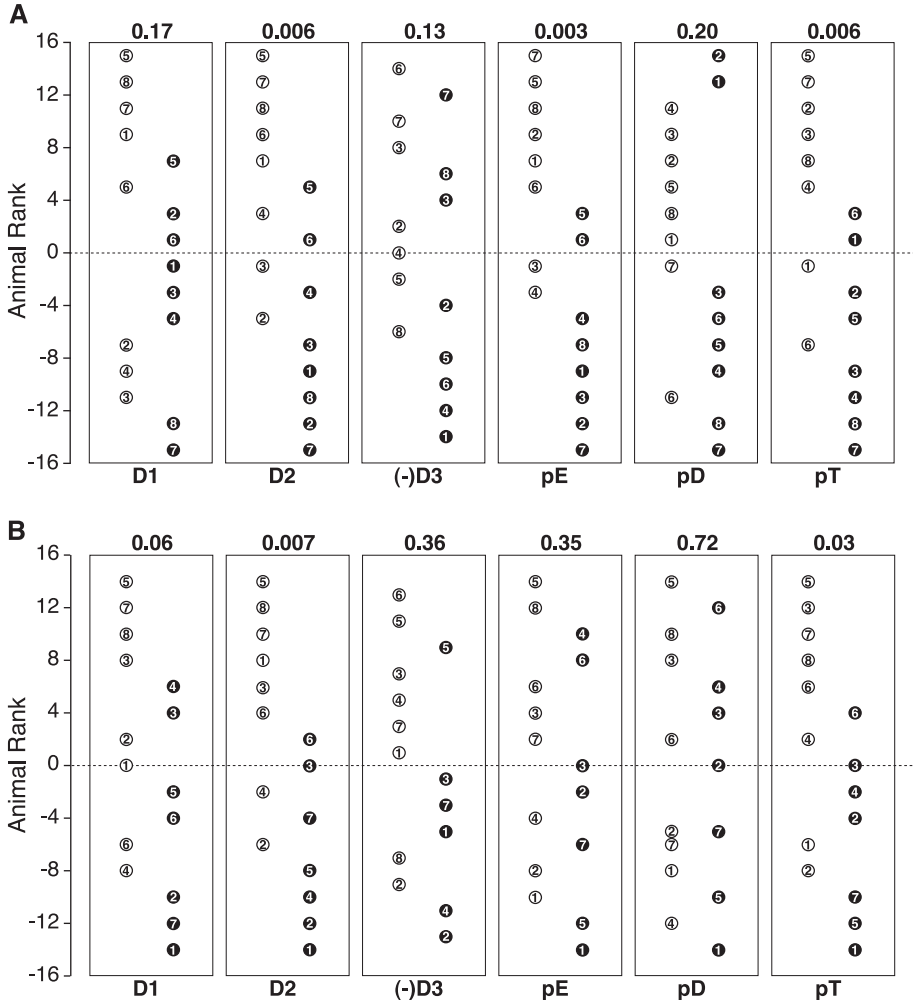

Fig. 2. Univariate analysis of gene expression in control rats (open circles with rat ID in black) and sucrose-dependent rats (filled circles with rat ID in white) in the nucleus accumbens (A) and the caudate-putamen (B). Abbreviations as in the legend to Fig. 1. D3 is displayed in the negative direction (rankings multiplied by −1) in order to conform all of the treatment effects to the same direction for the purpose of carrying out the multivariate analyses shown in Figs. 3 and 4. Wilcoxon–Mann–Whitney test p value is shown above each profile.

contributions from these two variants, which might have different functions [67].

2.3. Data analysis

Real-time PCR technology was used to measure mRNA expression for a number of genes previously studied in morphine-dependent rats [26,58]. The data analysis was carried out on log transformed data. Sample-to-sample variability was reduced by normalizing to levels of expression of genes determined to be house-keeping genes (S-100 beta, SNAP-25, synaptophysin, beta-actin, GAPDH and CaML). The average expression of all six of these genes was determined for each sample, and that expression level was used to "normalize" the samples before determining the relative levels of expression for the other genes of interest. Treatment-independent correlation between genes (Table 1) was computed by the Pearson correlation coefficient of the above data after subtracting within-treatment means. Statistical tests of the above data were based on *u* statistics.

To address possible interactions between genes, we modified a method for partial ordering originally developed within the framework of the marginal likelihood principle [71]. While the original algorithm has been successfully applied to a number of biological phenomena [5,20,53,63,72], its computational efficiency was low. *U* statistics allow a more efficient algorithm for computing

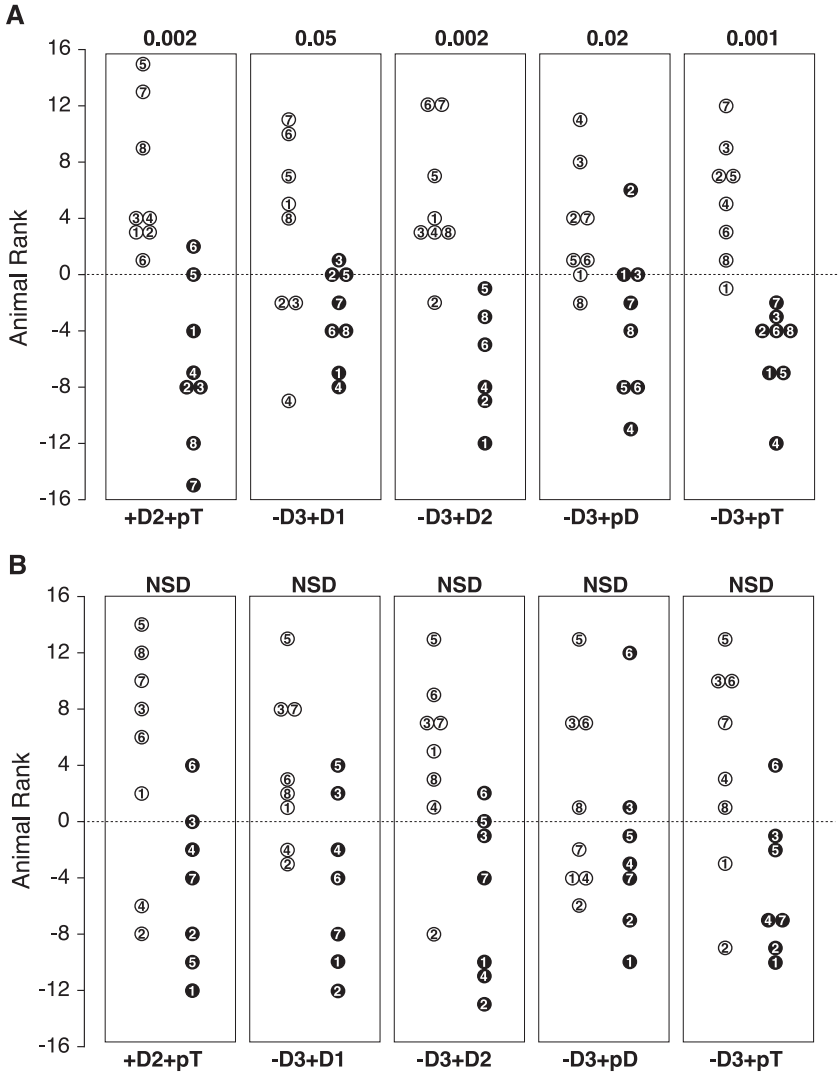

Fig. 3. Bivariate analysis of gene expression in sucrose-dependent and control rats in: the nucleus accumbens (A) and the caudate-putamen (B). Abbreviations as in the legend to Fig. 1. Wilcoxon–Mann–Whitney test *p* value is shown above each profile. Only the nucleus accumbens showed increased treatment group discrimination by bivariate analysis, and the rankings in the caudate-putamen are shown only for comparative purposes. The combinations with D3 were taken for D3 in the opposite direction, as indicated by the minus sign, showing that increases in D3 cooperate with decreases in the other genes. NSD indicates that the pairs did not improve the discrimination of the treatment groups at least threefold beyond that provided by the univariate analysis of either member of the pair. In the caudate-putamen, no pairs of genes significantly improved discrimination of the treatment effect.

scores for each profile of two or three genes [73]. The score for each sample profile was computed as the number of sample profiles that were lower minus the number that were higher than that sample [37]. Profiles were created from all pairs and trios. As it is not known a-priori whether particular genes will interact positively or negatively, a separate profile was created for each combination of signs (directions) that could be assigned to a set of genes, and the reported results are for the combination of signs that discriminates best. In the present study, all treatment effects except D3 were decreases in expression, and D3 interacted in a cooperative manner with other genes when its sign was changed (i.e., multiplied by minus one), while the other interactions were identified without changing signs. The resulting *u* scores were then analyzed by means of a Wilcoxon–Mann–Whitney-type score test [28]. For profiles based on a single gene, this reduced to the WMW test [39]. Multivariate orderings that improved group identification, by threefold over that provided by the members of the pairs or trios, were selected as significant if the *p* value was less than 0.05. A utility for carrying out multivariate analyses is available upon request from K.M.W., e-mail kmw@rockefeller.edu.

3. Results

3.1. Magnitude of treatment effects and univariate analysis

Fig. 1 shows the magnitude of alterations of mRNAs in sucrose-dependent rats relative to controls. The effects of sucrose dependency on gene expression, whether a decrease or an increase, were consistently greater in the nucleus accumbens than in the caudate-putamen. D2 and pE showed the largest differences, with a much greater decrease in the nucleus accumbens than in the caudate-putamen. As shown in the parallel dot graphs of Fig. 2A and B, illustrating all ranking scores, sucrose effects, by univariate analysis, reached statistical significance at the 0.05 level for D2, pE and pT in the nucleus accumbens, and for D2 and pT in the caudate-putamen.

3.2. Multivariate analyses

We explored the difference between the nucleus accumbens and caudate-putamen further, using the recently developed multivariate analysis method described in the Methods section. This multivariate analysis markedly improved the discrimination between treatment groups in the nucleus accumbens, while it did not do this for the caudate-putamen. Pairs of genes that interacted to identify treatment effects in the nucleus accumbens are plotted in Fig. 3A, while the corresponding pairs for the caudate-putamen, which did not improve identification of treatment groups, are plotted in Fig. 3B for purposes of comparison. The trios of genes that significantly improved the identification of sucrose-dependent rats in the nucleus accumbens are plotted in Fig. 4A,

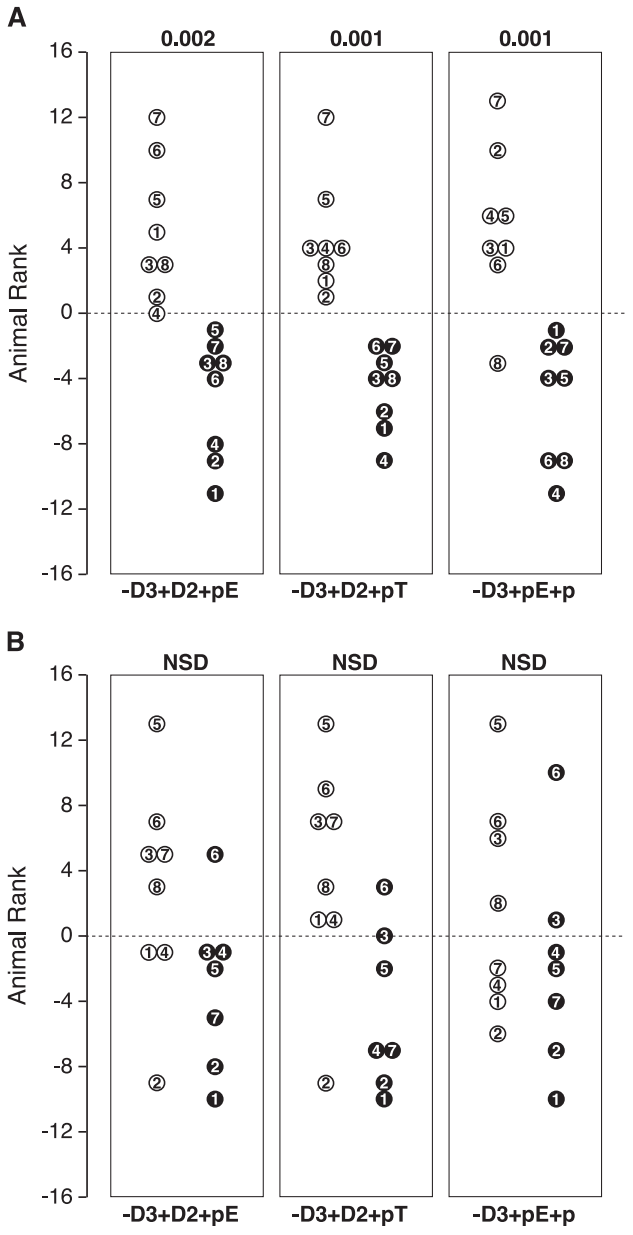

Fig. 4. Trivariate analysis of gene expression in sucrose-dependent and control rats in: the nucleus accumbens (A) and the caudate-putamen (B). Abbreviations as in the legend to Fig. 1. Wilcoxon–Mann–Whitney test *p* value is shown above each profile. Only the nucleus accumbens showed increased treatment group discrimination by trivariate analysis, and the rankings in the caudate-putamen are shown only for comparative purposes. The combinations with D3 were taken for D3 in the opposite direction, as indicated by the minus sign, showing that increases in D3 cooperate with decreases in the other genes. NSD indicates that the trios did not improve the discrimination of the treatment groups at least threefold beyond that provided by the univariate analysis of any member of the trio. In the caudate-putamen, no trios of genes significantly improved discrimination of the treatment effect.

while the corresponding (nonsignificant) trios in the caudate-putamen are plotted in Fig. 4B, for purposes of comparison.

From multivariate analyses, D3 in the nucleus accumbens was found to be the most important interactor in

bivariate (Fig. 3A) and trivariate (Fig. 4A) comparisons. When D3 (multiplied by −1 in order to conform the treatment effects to the same direction) was taken together with either D1, D2, pT or pD, there was a significant improvement in the ability to identify treatment effects in the nucleus accumbens (Fig. 3A). D3, in the negative direction, was included in all trivariate comparisons that improved identification of the treatment groups (Fig. 4A). The profile of −D3+pT discriminated treatment effects best among all pairs (Fig. 3A), and −D3+pT was included in two of the significant trivariate profiles (Fig. 4A). This suggests that D3 might share in mediating the treatment response with D1, D2, pT and pD.

3.3. Correlation analysis

To gain further insight into the nature of possible interactions between genes, we carried out a correlation analysis. This analysis, like the multivariate analysis, revealed clear differences between the nucleus accumbens and caudate-putamen (Table 1). In the nucleus accumbens, all genes were only moderately correlated, without any obvious pattern (interquartile range: 0.44–0.78). These over-all lower correlations in the nucleus accumbens contrasted with those observed in the caudate-putamen, where five genes (D1, D2, pE, pD and pT) were highly correlated with one another (0.83–0.95) but not with D3. D3 in the caudate-putamen was weakly correlated (negatively) with these genes (0.10–0.25). Thus, generally lower correlations were seen in the nucleus accumbens, where the multivariate analysis improved group discrimination, while generally higher correlations were seen in the caudate-putamen, where the multivariate analysis failed to reveal cooperative interactions.

Table 1
Correlation analysis in the nucleus accumbens (NAC) and caudate-putamen (CPU) after subtraction of the treatment effect

NAC	D1	D2	D3	pE	pD	pT
D1		0.92	0.55	0.78	0.32	0.67
D2			0.41	0.82	0.20	0.64
D3				0.47	0.78	0.71
pE					0.10	0.65
pD						0.77
pT						

CPU	D1	D2	D3	pE	pD	pT
D1		0.90		0.85	0.84	0.85
D2				0.89	0.87	0.89
D3	-0.10	-0.21				
pE			-0.16		0.95	0.90
pD			-0.13			0.90
pT			-0.25			

Abbreviations as in the legend to Fig. 1.

4. Discussion

These findings raise a number of important points for discussion, including the similarity of alterations in sucrose-dependent and morphine-dependent rats, as well as apparent differences between sucrose effects in the nucleus accumbens and adjacent caudate-putamen.

4.1. Opiate-like effects of sugar

A main finding of this study is that changes in mRNA levels in sucrose-dependent rats are similar to those previously identified in morphine-dependent rats. Both morphine and sucrose cause a reduction in D2 mRNAs [26,58,65], a reduction in opioid mRNAs [26,49,65,66,74], and an increase in D3 mRNA [58] in the striatal forebrain. This similarity indicates that sucrose and morphine might activate similar pathways, either directly in the forebrain, or in regions which project to the forebrain.

Some effects of morphine and sucrose might be mediated by common mechanisms, such as alterations in dopamine transmission. While the mechanisms by which palatable foods elevate forebrain dopamine are not clearly understood, one mechanism involves release of opioids in the area of midbrain dopamine neurons [18,23,36,56,62]. It is thought that the opiate, morphine, acting on opioid receptors on GABAergic neurons in the region of midbrain dopamine neurons, disinhibits firing of dopamine neurons, leading to increased dopamine release in the forebrain [19,30,34]. In support of a role for morphine-like activation of dopamine neurons in feeding behavior, direct injections of opioids in the midbrain enhance feeding [4,46]. This effect of morphine presumably mimics one of the effects of endogenous opioid release, which normally occurs to facilitate adaptive behaviors, such as eating [35]. If a common mediator of sucrose and morphine effects is dopamine transmission, then similar effects would be expected in the dopamine-receptive forebrain, as was seen in the present study. In support of this model, a recent investigation showed that the

transcription factor CREB, acting in the forebrain, modulates the rewarding effects of both sucrose and morphine [6]. One possible explanation for this, among many others, is that dopamine mediates some effects of both sucrose and morphine [1,7,8,11,15–17,22,43,47,48,75].

4.2. Differential effects in the striatum

Previous studies with morphine have reported similar effects on gene expression in the nucleus accumbens and caudate-putamen [26,40]. An important finding of the present study is that the effects of sucrose on gene expression, unlike the effects of morphine, differ between the nucleus accumbens and caudate-putamen. First, the magnitude of sucrose effects was consistently larger in the nucleus accumbens than in the caudate-putamen (Fig. 1), indicating that sucrose specifically targets cells in the nucleus accumbens, rather than having a generalized effect across the dopamine-receptive striatum. Second, multivariate comparisons of gene combinations significantly improved the ability to identify treatment effects only in the nucleus accumbens (Figs. 3 and 4), suggesting that the nucleus accumbens responds to sucrose in a qualitatively different way than the caudate-putamen. Third, correlations between genes in the nucleus accumbens were considerably lower than in the caudate-putamen (Table 1), supporting a load-sharing model to explain why multivariate comparisons were able to better identify treatment effects in the nucleus accumbens, as discussed further below.

The nature of the qualitative differences in gene–gene interactions between the two striatal areas suggests that some genes in the nucleus accumbens share the load of responding to sucrose treatment. Such cooperative load-sharing mechanisms regulate biochemical systems in many physiological contexts, providing redundant pathways to bring about a specific result [9]. If two or more genes share the response-load associated with bringing a system back to a homeostatic set-point, different animals can spread the response-load differently. In a load-sharing system, if some animals respond to a stimulus by activating pathway A, while others activate pathway B, then a single measure of ONLY A or ONLY B will be less effective at distinguishing the treatment groups than a combined measure of A AND B; on the other hand, a linear combination of A PLUS B may be overly simplistic.

The lower correlations between genes in the nucleus accumbens (Table 1) are consistent with the load-sharing model suggested by the results of the multivariate analysis (Figs. 3A and 4A). If two genes share a response-load, their expression will be less correlated, and the treatment effect will be better identified by a combination of the two genes. In contrast, a high degree of correlation between genes might indicate that there are, for example, high responders and low responders among the animals, rather than interactions between the response genes in individual animals. This type of co-regulation within animals would be associated with small gains of multivariate over univariate discrimination, as was seen in the caudate-putamen, where five genes were all highly correlated, while D3 was weakly correlated (negatively) with the other genes (Table 1). This suggests that, in the caudate-putamen, D3 plays an independent role, while the other genes measured are co-regulated. In the adjacent nucleus accumbens, however, D3 seems to interact with the other genes, perhaps in a load-sharing manner.

4.3. Summary and perspective

An animal model of intermittent bingeing on sugar produced opiate-like effects on striatal gene expression, especially in the nucleus accumbens. This model might provide a path to identifying specific neurons that are associated with responses to ‘‘reward’’. Presumably, these same neurons are also activated by drugs of abuse such as morphine, but only incidentally, as part of a wider, nonspecific activation of neurons in, for example, the dopamine-receptive fields. Some of these effects, including a decrease in D2 and an increase in D3, suggest a compensatory mechanism at work to mitigate the effects of abrupt excesses in dopamine that is released by eating the sugar. If true, these findings indicate that some similar differences associated with human diseases might not be causative, but rather compensatory. For example, decreased D2 in obese subjects might be a response to over-eating, rather than a difference that predisposes individuals to over-eat [68], and the increased D3 seen in cocaine abusers and schizophrenics might not represent a genetic disposition to disease, but rather a ‘‘healthy’’ counter-regulatory response, perhaps to hyperdopaminergia [7,42].

Acknowledgements

Our thanks to Paolo Giorno and RU Media Services for designing and refining the figures. This work was supported by funds from: a Burroughs-Wellcome Fellowship Award (NLG), General Clinical Research Center grant M01-RR00102 from the National Center for Research Resources (KMW); NIH grant DA10608 (BGH); and NIH grant MH 43422 (SFL).

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