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Molecular genetic responses to lysergic acid diethylamide include transcriptional activation of *MAP kinase phosphatase-1*, *C/EBP-β* and *ILAD-1*, a novel gene with homology to arrestins

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Abstract

We recently demonstrated that the potent hallucinogenic drug lysergic acid diethylamide (LSD) dynamically influences the expression of a small collection of genes within the mammalian prefrontal cortex. Towards generating a greater understanding of the molecular genetic effects of hallucinogens and how they may relate to alterations in behavior, we have identified and characterized expression patterns of a new collection of three genes increased in expression by acute LSD administration. These genes were identified through additional screens of Affymetrix DNA microarrays and examined in experiments to assess dose–response, time course and the receptor mediating the expression changes. The first induced gene, *C/EBP-β,* is a transcription factor. The second gene, *MKP-1,* suggests that LSD activates the MAP (mitogen activated protein) kinase pathway. The third gene, *ILAD-1*, demonstrates sequence similarity to the arrestins. The increase in expression of each gene was partially mediated through LSD interactions at 5-HT_{2A} (serotonin) receptors. There is evidence of alternative splicing at the *ILAD-1* locus. Furthermore, data suggests that various splice isoforms of *ILAD-1* respond differently at the transcriptional level to LSD. The genes thus far found to be responsive to LSD are beginning to give a more complete picture of the complex intracellular events initiated by hallucinogens.

Keywords: gene expression, hallucinogen, prefrontal cortex, serotonin receptors, synaptic plasticity.

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Hallucinogenic drugs such as lysergic acid diethylamide (LSD) have profound effects on human consciousness, including disruption of sensory processing, alterations of various cognitive processes and can produce hallucinations and loss of contact from reality. Recently, parallels have been emphasized between the effects of hallucinogens and early symptoms of schizophrenia (Vollenweider *et al*. 1997; Aghajanian and Marek 2000). Although the actions of hallucinogens are believed to be mediated primarily by serotonin receptor activation (Krebs-Thomson *et al*. 1998; Marek and Aghajanian 1998; Winter *et al*. 2000), attempts to understand the entire process from receptor–ligand interaction to behavior have led only to modest advances, despite more than four decades of investigation.

Most recent studies into the mechanisms of action of hallucinogens have focused on acute intracellular and electrophysiological events. For example, Aghajanian and Marek (Aghajanian and Marek 1999a,b) have reported that the application of hallucinogens to cortical pyramidal cells facilitates late asynchronous EPSPs mediated by release of glutamate in response to 5-HT_{2A} receptor activation. These effects are not observed when the endogenous ligand serotonin (5-HT) is applied, apparently due to masking by opposing effects at non-5-HT_{2A} receptors, most likely of the 5-HT_{1A} type (Araneda and Andrade 1991; Martin-Ruiz *et al*. 2001). In addition, hallucinogens may differentially activate various signal transduction cascades that are coupled to serotonin receptors. Backstrom and co-workers have shown that LSD activation of 5-HT_{2C} receptors induces signal

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Abbreviations used: 5-HT, 5-hydroxytryptamine or serotonin; DRH1, down-regulated in advanced human hepatocellular carcinoma; ERK, extracellular signal-regulated kinase; FAM, 6-carboxyfluorescein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ILAD-1, induced by lysergic acid diethylamide; JNK, c-Jun N-terminal kinase; LSD, lysergic acid diethylamide; MAP kinase, mitogen activated protein kinase; TAMRA, *N*,*N*,*N*′,*N*′-tetramethyl-6-carboxyrhodamine; TXNIP, thioredoxin-binding protein-2/vitamin D3 up-regulated protein-1.

transduction events different from the endogenous ligand serotonin (Backstrom *et al*. 1999). Similarly, Kurrasch-Orbaugh and co-workers have shown that hallucinogens may differentially activate transduction cascades that are coupled to the 5-HT_{2A} receptor (Kurrasch-Orbaugh *et al*. 2003b). Ultimately, these acute cellular events will influence gene expression, which has the to potential to alter the physiological properties of neurons on a long-term basis.

Recently, we showed that LSD produces robust and dynamic changes in gene expression within the prefrontal cortex (Nichols and Sanders-Bush 2002; Nichols *et al*. 2003). Many of these genes have previously been implicated in the process of synaptic plasticity. It is quite possible that these changes in gene expression have long-term neuromodulatory effects and contribute to behavioral responses to hallucinogens. Such changes may be especially relevant to a particular set of temporally delayed effects of LSD in humans, as defined by Freedman (1984), that are similar to certain aspects of schizophrenia that include paranoia and thought disturbances. Our first screen of the Affymetrics U34A microarray, containing probe sequences representing around 8000 genes, found surprisingly few gene expression changes. To identify additional genes influenced by LSD that were possibly missed in our first screen, we re-screened the U34A array. To identify novel candidates, we performed an initial screen of the Affymetrix U34B EST array, which contains probe sequences representing around an additional 8000 genes. The RNA used for these new screens was isolated from the prefrontal cortex of rats treated identically to those in our first study.

Materials and methods

Materials

(+)-LSD tartrate was obtained from NIDA (Baltimore, MD, USA). MDL100 907 (Johnson *et al*. 1996) was obtained from Hoechst Marion Roussel (Cincinnati, OH, USA). WAY100 635 (Critchley *et al*. 1994) was obtained from RBI (St Louis, MO, USA). Tri-Reagent® was from Molecular Research Center (Cincinnati, OH, USA). The RPA III™ kit and MaxiScript™ kit were purchased from Ambion (Austin, TX, USA). [^{32}P]CTP was purchased from Amersham (Piscataway, NJ, USA). TaqMan® Rodent GAPDH Control Reagents and TaqMan® One Step RT-PCR Master Mix Reagents kit were purchased from Applied Biosystems (Foster City, CA, USA). TaqMan® probe was synthesized by Qiagen Operon (Alameda, CA, USA) with 5′-conjugated FAM and 3′-conjugated TAMRA. PCR primers were synthesized in the Vanderbilt Molecular Biology Core Facility.

Animals

Male Sprague-Dawley rats (250–275 g) were purchased from Harlan and maintained for at least 1 week prior to use. Rats were given *ad libitum* access to food and water and maintained on a 12-h light/dark cycle. All procedures were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH publication no. 8023, revised 1978) and were approved by the Vanderbilt Animal Care and Use Committee.

Drug treatment and tissue preparation

(+)-LSD tartrate was dissolved at a concentration of 1.0 mg/mL in sterile water. MDL100 907 was dissolved at a concentration of 1.0 mg/mL in sterile water. WAY100 635 was dissolved at a concentration of 10.0 mg/mL in sterile water. For the antagonist studies, MDL100 907 (1.0 mg/kg) or WAY100 635 (10 mg/kg) was administered i.p. 30 min prior to LSD. Ninety minutes post LSD administration the animals were decapitated. For the time course experiments, 1.0 mg/kg of LSD was administered and the animals decapitated after 45, 90, 180 and 300 min. For the dose–response experiments, LSD was administered i.p. in doses of 0.20 mg/kg, 0.5 mg/kg and 1.0 mg/kg and the tissue collected at 90 min. The brains were removed and the appropriate tissues were quickly dissected, frozen in liquid nitrogen and stored at −80°C until processing. For the regional studies, prefrontal cortex, hippocampus and mid-brain regions were used. For each of the other studies (time–course, dose–response, antagonist), prefrontal cortex only was used. Total RNA was extracted using Tri-Reagent® and was used as target in RNase protection assays and as template in quantitative RT-PCR.

RNA samples and expression testing

The 1.0 mg/kg LSD time (t) = 90 min and matched control RNAs were gathered in three independent experiments. In each of these experiments, the RNA was purified from four pooled prefrontal cortexes per treatment group. The RNA from each experiment was tested separately and the data for this time point as shown in Figs 2 and 3 represent the average of the results for each experiment. For each of the other experiments and data points, except for the antagonist studies, the RNA purified from each of four rats was kept separate and not pooled for each treatment, representing different doses and times (except for the t = 300 groups, which used three rats per treatment group). For the TaqMan experiments, each individual sample of RNA was tested in three to four independent amplification reactions and the results averaged. These numbers were then used to generate the final expression values by averaging for each treatment condition. For the antagonist studies, the RNA from the prefrontal cortex of four animals pooled was used per treatment group. Each RNA sample from the pooled tissues was tested by RNase protection assay in three independent hybridization reactions.

Microarray screens

Total RNA was isolated from the prefrontal cortex of rats treated with LSD (1.0 mg/mL, t = 90 min) and control rats as previously described (Nichols and Sanders-Bush 2002) and sent to Genome Explorations (Memphis, TN, USA) for screening against the Affymetrix (Santa Clara, CA, USA) rat U34A and U34B genome microarrays. Analysis was performed by Genome Explorations using Microarray Suite 5.0 (Affymetrix) to identify significant changes in expression with at least a twofold difference between control and LSD treatment groups.

RNase protection

RNase protection was utilized in the initial verification of candidate genes and in the antagonist studies. The RPA III™

Table 1 Primer sequences used to generate probe template for RNase protection assays

RPA	Forward primer	Reverse primer
C/EBP	TCGGGACTTGATGCAATCCG	GCAGGAACATCTTTAAGTGATTACTC
MKP-1	GTGTGCCTGACAGTGCAGAATC	ATCAAAGCAGTGATACCCAAGG
ILAD-1 (3′)	ATCTGCCATCCATGTTCAGAACC	CAAAAGTGGTCCATTCTTCAGACC
ILAD-1 (exon IV/V)	AGTGGCACGATCCTGGTACTGC	GGCCATGAAGGTCTGTGTCTGTAC

kit reagents and protocols from Ambion were used as described previously (Nichols and Sanders-Bush 2002). Gene specific primer sequences to generate probe template via RT-PCR, as previously described (Nichols and Sanders-Bush 2002), are listed in Table 1. Probe was synthesized and labeled with [^{32}P]CTP using the MaxiScript kit from Ambion. Total probe used in each reaction was 70 000 c.p.m. for gene specific RNA and 1400 c.p.m. for the internal standard, cyclophilin, with 10 μg of total RNA per reaction. After electrophoresis, gels were dried on Whatman paper and exposed to phosphoimager plates (Molecular Dynamics, Sunnyvale, CA, USA). Bands were visualized using either a Molecular Dynamics 445 SI Phosphoimager or Typhoon 9400 Phosphoimager (Amersham Biosciences). Band densitometry analysis was performed with NIH Image 1.6.1 software on MacOS. (http://rsb.info.nih.gov/nih-image)

Real time RT-PCR

Gene expression levels in all experiments, except initial verification and antagonist studies, were performed utilizing real-time reverse transcription PCR using the ABI Prism 7700 and associated reagents (Applied Biosystems). This procedure is also known as the TaqMan® assay and measures real-time fluorescence accumulation of a reporter dye released from its quenched position on a gene specific DNA probe during incorporation into the amplification product. GAPDH amplified in the same reaction using a different fluorophore (TaqMan® Rodent GAPDH Control Reagents, ABI) was used as an internal standard to normalize between samples. Gene specific primer and probe sequences were determined using Primer Express 1.5 (Applied Biosystems) for Mac OS9 and are listed in Table 2.

Assay

The TaqMan® One Step RT-PCR Master Mix Reagents kit was used to perform one tube RT-PCR and amplifications in a 96-well format. Total RNA (10 ng) was used per reaction. Primer concentrations were 100 nM and a probe concentration of 250 nM per reaction was used for gene specific and GAPDH reagents. Cycle parameters were: 30-min RT at 48°C, 10-min denature at 95°C, 40 cycles of 15 second denature at 95° and 1-min anneal/extension at 60°. Data were gathered and formatted using SDS 1.9 (Applied Biosystems) on Mac OS9. Relative quantification of expression levels was determined using the C_T method as described by Applied Biosystems (User Bulletin #2, ABI Prism 7700 Sequence Detection System, 10/2001).

Results

Candidate genes identified

The Affymetrix U34A and U34B Rat microarrays were screened and yielded a total of 11 primary candidates for increased gene expression. Each of these was tested by RNase protection with prefrontal cortex RNA to validate differential expression. Of these, four genes from the U34A set were confirmed: *IKβ-α*; *serum glucocorticoid kinase* (*sgk*); *CCAAT enhancer binding protein β* (*C/EBP β*); and *map kinase phosphatase-1* (*MKP-1*). The first two genes, *IKβ-α* and *sgk*, were also identified in our earlier screen (Nichols and Sanders-Bush 2002), whereas the latter two genes were newly identified. The screen of the U34B microarray yielded one confirmed up-regulated gene represented by EST192132, now called *induced by lysergic acid diethylamide-1* (*ILAD-1*). The fact that there was only a two-gene overlap between the first and second rounds of screening the U34A microarray suggests that further re-screens of both microarrays may identify additional LSD-responsive genes. The remainder of this report focuses on the three new up-regulated genes.

Expression in different areas of the brain

Expression in the prefrontal cortex for *C/EBP*, *MKP-1* and *ILAD-1* was induced approximately twofold by 1 mg/kg LSD at 90 min (Fig. 1). In the hippocampus, gene expression of *C/EBP* was not significantly altered, while *MKP-1* and *ILAD-1* were still increased by about twofold (Fig. 1). Within the midbrain region, *C/EBP* was increased by approximately 50%, while *MKP-1* and was increased about twofold (Fig. 1).

Table 2 Primer and probe sequences used for quantitative RT-PCR

TQM	Forward primer	Reverse primer	Probe
C/EBP	GGGACTTGATCGAATCCGG	GTTGCGTCAGTCCCGTGTC	TCAAACGTGGCTGAGCGCGTG
MKP-1	TTGAGTCCCAAGTACTGGCCC	AAGGTCAAGGACAGCCAT	CTGCAGAAGCTGGGAGCCCGG
ILAD-1	GGCCCAAGGACTGGTGGT	GGTTCTGAACATGGATGGCAG	CAGATGAGCCCAGAACTGTGGTTGTGA

TaqMan probe sequences were conjugated to the reporter dye FAM at the 5′ end, and the quencher TAMRA at the 3′ end.

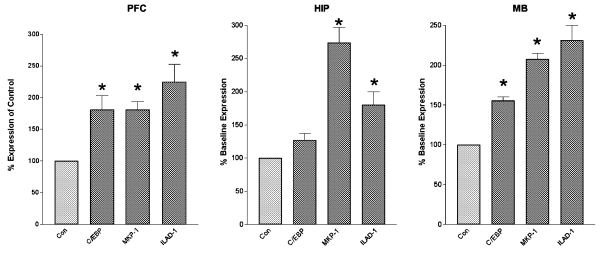

Fig. 1 Gene expression in different brain areas. Expression levels altered by LSD (1.0 mg/kg, $t = 90$ min) are shown for each gene in the prefrontal cortex (PFC), hippocampus (HIP) and midbrain (MB) as determined in real-time PCR experiments (*$p < 0.05$ vs. control; Student's *t* test).

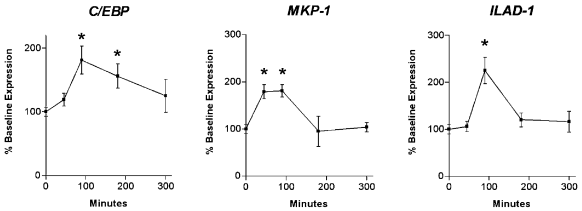

Fig. 3 Time–course. The time–course of expression for each gene at various time points after LSD (1.0 mg/kg LSD) in the prefrontal cortex was determined by real-time PCR experiments. *MKP-1* responds very early. Each returns to baseline rapidly with the exception of *C/EBP*, which decreases more slowly over 5 h (*$p < 0.05$; two-way ANOVA with four comparisons).

Dose–response

Expression was examined at four treatment conditions, each at 90 min: 0 mg/kg, 0.2 mg/kg, 0.5 mg/kg and 1.0 mg/kg LSD. The *C/EBP* and *ILAD-1* transcripts were observed to be near maximally induced at the lower dose of 0.5 mg/kg (Fig. 2). *MKP-1* expression increased through 1.0 mg/kg (Fig. 2).

Time course

The time course of expression was examined at 0, 45, 90, 180 and 300 min after 1.0 mg/kg LSD. The maximum increase of *MKP-1* occurred quite early, at 45 min and returned to baseline levels by 3 h (Fig. 3). The expression of *C/EBP* peaked at 90 min and slowly decreased to baseline levels over 5 h (Fig. 3). *ILAD-1* expression was very dynamic, remaining at baseline for 45 min, quickly peaking by 90 min and returning to baseline levels by 3 h (Fig. 3).

Antagonist studies

The effects of the selective 5-HT_{2A} antagonist MDL100 907 were examined on gene expression (Figs 4 and 5). Pre-treatment with 1.0 mg/kg partially blocked the LSD-induced expression increases for all three transcripts, with *C/EBP* being blocked the most, but not completely. The effects of pre-treatment with the selective 5-HT_{1A} receptor antagonist

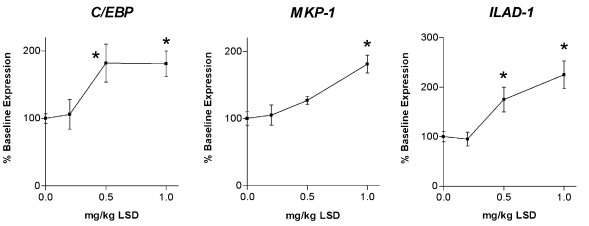

Fig. 2 Dose–response. The dose–response to LSD at $t = 90$ min in the prefrontal cortex is shown as determined by real-time PCR experiments. Doses examined were 0.2, 0.5 and 1.0 mg/kg LSD. *C/EBP* and *ILAD-1* respond to lower doses of drug (*$p < 0.05$; one-way ANOVA with simple comparisons).

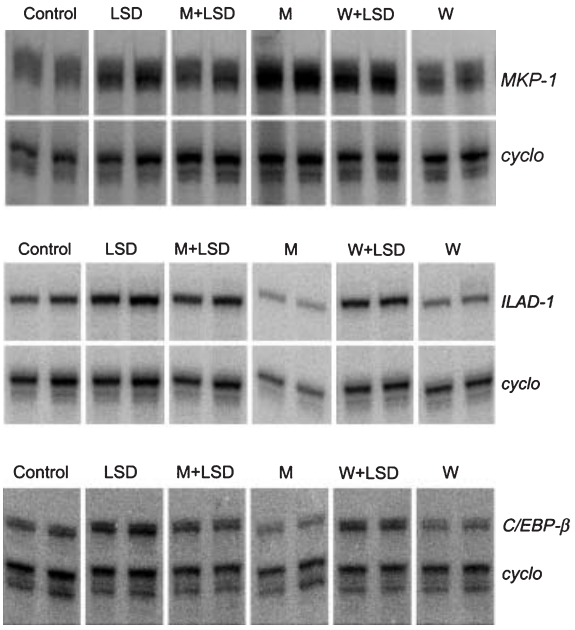

Fig. 4 RNase protection analysis. This figure shows representative data from the Rnase protection analysis experiments for the antagonist studies. The upper band signal in each represents the specific gene tested and the lower band represents the internal control *cyclophilin* (*cyclo*). Control, no treatment; LSD, treatment with 1.0 mg/kg LSD 90 min; M, 5-HT_{2A} antagonist MDL100907 (1.0 mg/kg) alone; M+LSD, pre-treatment with MDL100907 30 min prior to LSD; W, 5-HT_{1A} antagonist WAY100635 (10 mg/kg) alone; W+LSD, pre-treatment with WAY100635 30 min prior to LSD.

WAY100 635 on gene expression was also examined. LSD-induced expression changes were not blocked by WAY100 635 for any of the transcripts (Figs 4 and 5).

Gene structure analysis of *ILAD-1*

A search of the GenBank database (http://www.ncbi.nlm.nih.gov/) with the EST192132 Affymetrix probe sequence highlighted an 82% region of identity over a 300-nucleotide region within the 3′ UTR of a mouse mRNA that was

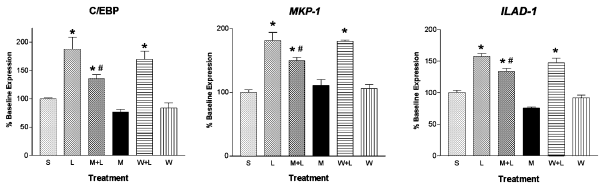

Fig. 5 Antagonist study results. The effects of pre-treatment with the 5-HT_{2A} receptor antagonist MDL100907 (M) or the 5-HT_{1A} receptor antagonist WAY100635 (W) in the prefrontal cortex were examined by RNase protection analysis. Expression increases for all three genes have a partial 5-HT_{2A} receptor-mediated component. [*Significantly different than S (one-way ANOVA with seven planned comparisons), #, pre-treatment + LSD sample significantly different than LSD alone (one-way ANOVA with seven planned comparisons)]. S, control; L, LSD alone; M+L, MDL100907 pre-treatment + LSD; M, MDL100907 alone; W+L, WAY100635 pre-treatment + LSD, W, WAY100635 alone.

partially sequenced as part of a large scale sequencing project (GenBank accession #BC054826) (Strausberg *et al*. 2002). The general structure of the rat *ILAD-1* gene was determined by comparing rat genomic sequences within the NCBI database to the previously partially sequenced orthologous mouse mRNA and to the predicted rat and mouse mRNAs (GenBank accession #XM_224720 and #AK014582, respectively). The full-length transcript is predicted to be approximately 2.3 kb, consisting of eight putative exons and coding for a predicted 407-amino acid open reading frame (Fig. 6a).

The predicted rat ILAD-1 protein has regions of high similarity to the arrestin family of proteins and contains both N-terminal and C-terminal arrestin domains as determined by analysis using Pfam (http://www.sanger.ac.uk/Software/Pfam/) (Fig. 6b). The N-terminal domain shows 40% identity and around 80% similarity over 130 amino acids to the Pfam consensus Arrestin-N domain sequence, while the C-terminal domain shows 36% identity and around 80% similarity to the Pfam consensus Arrestin-C domain sequence over 127 amino acids (Fig. 6b). There is a putative human ortholog of ILAD-1 within the GenBank database that shows 82% identity and 92% similarity with the rat primary nucleic acid sequence.

It is interesting to note that the orthologous mouse *ILAD-1* mRNA partial sequence within the GenBank database contains predicted intron IV sequence, as well as other intronic sequences. Because the sequenced mRNA and predicted mRNA differed in a way to suggest the presence of alternative splicing, RT-PCR was performed using rat sequence specific primers encompassing the boundary

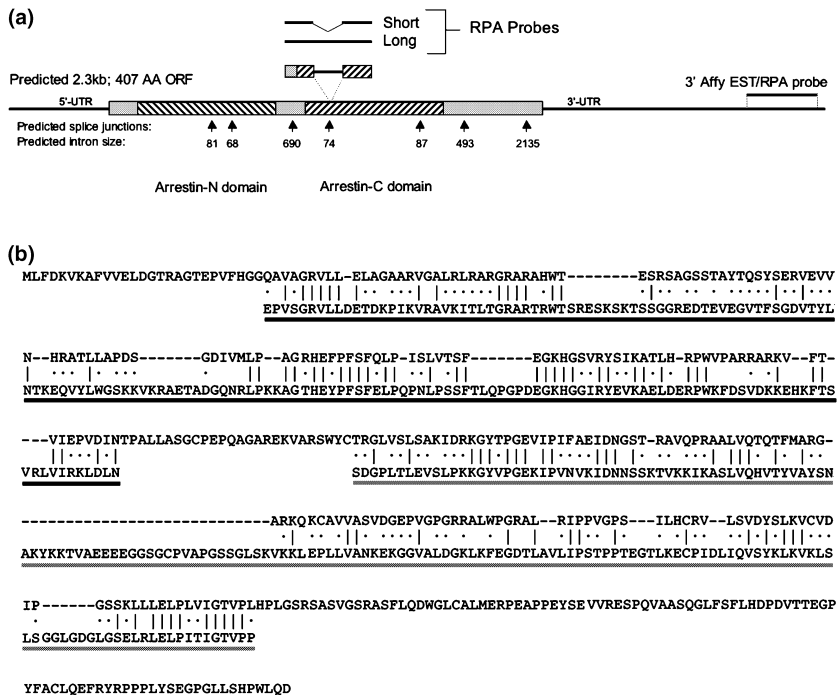

Fig. 6 Predicted structure of *ILAD-1* and protein. (a) The predicted structure of the rat 2.3-kb *ILAD-1* gene as determined by comparing genomic sequences within the public NCBI database with sequenced and predicted mRNAs. The 407 AA ORF is represented by the boxed region, and the UTRs by the lines on either side of the boxed region. The arrestin homology domains are shown as cross-hatched boxes. The location of the Affymetrix probe sequence is shown on the 3′ end. This region was used to generate the real-time PCR amplicon, as well as RPA probe sequences for the antagonist studies. Probes generated to perfom RPA analysis on putative splice isoforms are indicated. (b) The predicted *ILAD-1* amino acid sequence. Pfam consensus sequences and Pfam alignments for the Arrestin-N (black underline) and Arrestin-C (grey underline) domains are shown. Identities are shown with a connecting line and similarities with a dot.

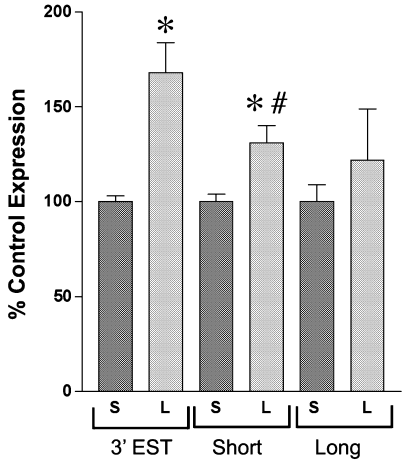

Fig. 7 Results of RNase protection analysis comparing different probe regions. The LSD-induced expression of each *ILAD-1* region tested, as highlighted in Fig. 6(a), are shown. The expression increase observed with the 3′ probe is around two-fold higher than the short, exon IV/V probe. This suggests that LSD can elicit different transcriptional responses from the same locus between putative splice isoforms. Results represent the average of three independent groups of animals tested for each treatment group, with each group comprised of the RNA from four animals pooled (*$p < 0.05$, S vs. L; #, $p < 0.05$, L-short vs. L-3′EST; two-factor ANOVA).

between exons IV and V to investigate. RT-PCR revealed two products, corresponding in size to the two possible splice isoforms with respect to included intron IV sequences (data not shown). RPA probes were generated towards the two putative isoforms and tested for LSD-induced expression within the prefrontal cortex along with the original 3′ probe fragment corresponding to the Affymetrix probe sequence (Fig. 6a). Each isoform-specific probe gave the predicted sized RPA hybridization signal and the results showed that the 3′ probe fragment had a roughly twofold greater response to LSD than the internal short isoform probe (Fig. 7). There is a trend for the unspliced isoform (Long) to be somewhat increased over baseline, however, exact conclusions cannot be drawn from this set of data because the results are not statistically different than either the control or the short isoform (Fig. 7).

Discussion

The re-screen of the Affymetrix U34A and initial screen of the U34B rat DNA microarrays identified 11 potential candidate genes whose expression increased in response to LSD within the prefrontal cortex. Two were previously identified as increased in expression by LSD, IKβ-α and *sgk* (Nichols and Sanders-Bush 2002). Each of the remaining candidates was tested by RNase protection to verify differential expression. Of these, it was confirmed that the expression of three additional genes was increased by LSD. The three new confirmed genes shown to be increased in expression are: *CCAAT enhancer binding protein β* (*C/EBP-β*), *map kinase phosphatase-1* (*MKP-1*) and *induced by lysergic acid diethylamide-1* (*ILAD-1*). These results are consistent with our previous screen in which approximately 30% of primary candidates identified by microarray analysis software were subsequently confirmed. Also consistent with our first screen, the difference in expression detected by Affymetrix software was often much greater than that determined by other more accurate methods such as RNase protection (Table 3). The general change in expression observed for these genes in the prefrontal cortex was around twofold. While the dose of LSD chosen to perform the microarray screens was high relative to amounts routinely used in behavioral studies, we believe that these results reflect relevant gene expression changes. Because the entire prefrontal cortex was used to prepare RNA for these experiments, there may have been discreet regions of high expression changes at lower doses of the drug that were masked by our use of bulk tissue. We anticipate that future investigations utilizing *in situ* techniques on the more interesting of these genes will highlight such expression changes at behaviorally relevant doses.

C/EBP-β, a transcription factor, belongs to a family of leucine zipper transcription factors involved in the regulation of cell growth and differentiation in many different tissues that are the endpoints for numerous signaling cascades. Among the processes in which C/EBP-β is specifically known to be involved are adipogenesis, cell cycle control and programmed cell death (reviewed in McKnight 2001).

Table 3 Genes identified as influenced in expression by LSD (1.0 mg/kg; 90 min)

Gene	Microarray	RNase protection	TaqMan
C/EBP	+400%	+188 ± 21%	+164 ± 11%
MKP-1	+260%	+179 ± 15%	+158 ± 8%
ILAD-1	+200%	+164 ± 12%	+205 ± 28%

The difference in expression found in the microarray analysis using Microarray suite 5.0 between the control and LSD prefrontal cortex samples is compared with that determined by experiments using RNase protection and real-time PCR (TaqMan). Expression increases as determined by microarray analysis are generally greater than either RNase protection or TaqMan expression results. The expression changes observed between RNase protection and TaqMan are generally similar. Values are percentage of control expression.

C/EBP β has been found to be expressed in mammalian brain and shown to promote neuronal differentiation and neurite outgrowth through the PI3K pathway (Cortes-Canteli *et al*. 2002). Taubenfeld and co-workers have shown that consolidation of new memories requires CREB-dependant C/EBP β activation in the hippocampus (Taubenfeld *et al*. 2001).

Map kinase phosphatase-1, also known as CL100, is a nuclear localized dual specificity protein phosphatase. It interacts with a variety of MAP kinases including ERK1, ERK2, JNK1 and p38α (Slack *et al*. 2001). The promoter region for the *MKP-1* gene includes binding site sequences for both AP1 (c-fos/c-jun heterodimers) and CRE transcription factors (Kwak *et al*. 1994). Its expression within the brain is localized to discrete areas that include high expression in specific regions of the cortex and thalamus (Kwak *et al*. 1994). Interestingly, the *MKP-1* transcript is rapidly increased after both a single dose of methamphetamine and behavioral sensitization to methamphetamine in the rat prefrontal cortex (Ujike *et al*. 2002). Its expression peaked very rapidly, at 30 min and then declined rapidly after acute administration of methamphetamine (Ujike *et al*. 2002) similarly to what was observed with LSD in the present experiments. The sensitivity of *MKP-1* to methamphetamine suggests that its LSD-influenced expression may be partially increased through LSD interactions at dopamine receptors, in addition to its partial effects at 5-HT_{2A} receptors as shown in this work (Figs 4 and 5). Because *MKP-1* transcription is mediated by the activation of MAP kinase cascades, which include SAPK/JNK and p38 (Bokemeyer *et al*. 1996), it is reasonable to assume that acute LSD rapidly activates the MAP kinase pathway. This conclusion is consistent with Kurrasch-Orbaugh *et al*. (2003a) who showed that activation of the 5-HT_{2A} receptor leads to phospholipase A2 activation through a complex signaling pathway involving MAP kinases. *MKP-1* was the only gene observed in our tests not to be induced at lower doses of LSD. However, in the absence of a full dose–response curve for this gene, which was seen to be increasing in expression through the highest dose, and without knowledge of the absolute levels of expression, we cannot make definite conclusions about the relative expression of *MKP-1* to the other two genes.

The third up-regulated gene identified in this study, and also one that is extremely interesting, is *ILAD-1*. It shows LSD-mediated increases in expression in all regions of the brain investigated and shows partial mediation through the 5-HT_{2A} receptor. The sequence of the predicted ILAD-1 protein shows similarity to the arrestin family of proteins (Fig. 6). While the homology to arrestins may be significant for the proteins function, there are higher degrees of overall similarity between ILAD-1 and thioredoxin-binding protein-2/vitamin D_3 up-regulated protein-1 (TXNIP/VDUP1) (Chen and DeLuca 1994; Nishiyama *et al*. 1999) and to down-regulated in advanced human hepatocellular carcinoma (DRH1) (Yamamoto *et al*. 2001). Sequence analysis indicates that both TXNIP and DRH1 also contain arrestin homology domains, but each has a slightly lesser degree of similarity to the Pfam arrestin domain consensus sequences than ILAD-1 has. Arrestins are involved in the processes of G-protein coupled receptor desensitization and internalization, and are necessary for many cellular functions (reviewed in: Ferguson 2001). TXNIP binds to and inhibits the reducing activity of thioredoxin (Nishiyama *et al*. 1999), which is one of the major thiol reducing systems and contributes to many cellular processes ranging from cell cycle control to apopotosis, and is regulated by a number of various stimuli (Nishinaka *et al*. 2001). The function of DRH1 remains unknown. While ILAD-1 is clearly related to arrestins, its exact function cannot be deduced at the present time. However, because both arrestins and TXNIP directly interact with other proteins, it is possible that the two arrestin domains confer a similar protein–protein interaction function to ILAD-1.

Differences between the sequences of a partially sequenced orthologous mouse mRNA and the predicted mRNA suggested that alternative splicing was occurring within the ILAD-1 locus. RT-PCR and RPA experiments using rat primer and probe sequences confirmed alternative splicing in at least one location around exon IV/V. Furthermore, RPA studies demonstrated that probes corresponding to different regions of the transcript had differing responses to LSD. For example: probe corresponding to the 3′ region of the gene had a roughly twofold greater response to LSD than probes corresponding to the exon IV/V region (Fig. 7). Together, these data raise the very interesting and exciting prospect of LSD treatment leading to different genetic responses from the same locus.

Each gene tested had its LSD response only partially mediated through 5-HT_{2A} receptor activation. None of these genes had even a partial 5-HT_{1A} receptor component. While it has been convincingly demonstrated that 5-HT_{2A} receptor activation is necessary for most behavioral effects of hallucinogens in animal models, it does not necessarily imply that genes without a major 5-HT_{2A} component do not play a role in hallucinogenic behaviors. In humans, the effects of different hallucinogens can be quite variable. It seems likely that some of these variations may be due to differences in the overall receptor binding profiles between different molecules. LSD is unique among hallucinogens in both its potency and behavioral profile in humans (Freedman 1984), and has affinity for a large number of GPCRs (Nichols *et al*. 2002; Roth *et al*. 2002), any one of which may mediate gene expression changes or LSD-specific behaviors.

In conclusion, we have now identified and characterized the expression patterns of 11 genes that are significantly increased in expression by LSD in mammalian prefrontal cortex. A common theme among these genes, including two of the genes identified in this study, *C/EBP and MKP-1*, is

the process of synaptic plasticity. It is likely that further microarray screens will identify additional genes, but in general the transcriptional response to the powerful hallucinogenic drug LSD appears to be relatively low. Interestingly, we now have evidence that response to LSD from a specific gene locus may be isoform specific. It is worth noting that apparently minor changes in brain neurochemistry have the ability to produce such profound and sometimes long-lasting effects in humans. It remains to be determined which receptors mediate some or all of the expression changes seen with many of the genes identified in these screens. It may be non-5-HT_{2A} or 5-HT_{1A} mediated changes, however, that point to novel mechanisms underlying LSD-specific behaviors. Continued research into the molecular genetic effects of LSD and other hallucinogens may provide clues to genetic regulation mechanisms that may be relevant to psychiatric disorders such as schizophrenia.

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