# The Effects of Hallucinogens on Gene Expression

**David A. Martin and Charles D. Nichols**

**Abstract** The classic serotonergic hallucinogens, or psychedelics, have the ability to profoundly alter perception and behavior. These can include visual distortions, hallucinations, detachment from reality, and mystical experiences. Some psychedelics, like LSD, are able to produce these effects with remarkably low doses of drug. Others, like psilocybin, have recently been demonstrated to have significant clinical efficacy in the treatment of depression, anxiety, and addiction that persist for at least several months after only a single therapeutic session. How does this occur? Much work has recently been published from imaging studies showing that psychedelics alter brain network connectivity. They facilitate a disintegration of the default mode network, producing a hyperconnectivity between brain regions that allow centers that do not normally communicate with each other to do so. The immediate and acute effects on both behaviors and network connectivity are likely mediated by effector pathways downstream of serotonin 5-HT2A receptor activation. These acute molecular processes also influence gene expression changes, which likely influence synaptic plasticity and facilitate more long-term changes in brain neurochemistry ultimately underlying the therapeutic efficacy of a single administration to achieve long-lasting effects. In this review, we summarize what is currently known about the molecular genetic responses to psychedelics within the brain and discuss how gene expression changes may contribute to altered cellular physiology and behaviors.

**Keywords** Psychedelics · 5-HT2A · IEG · Gene expression

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## 1 Introduction

The immediate behavioral effects that result from the administration of classic hallucinogens, or psychedelics, occur through activation of 5-$HT_{2A}$ receptors and subsequent changes in synaptic transmission and action potential firing. Alterations in subjective experience as well as changes in neuronal activity are seen in a matter of seconds following the intravenous injection of psychedelics (Strassman et al. 1994; Carhart-Harris et al. 2011; Riga et al. 2014). Psychedelics and many other psychotropics also initiate neuronal signaling that occurs over longer intervals. This signaling includes alterations in patterns of mRNA expression in cells activated by the drug, along with corresponding alterations in protein translation. Lysergic acid diethylamide (LSD) and 2,5-dimethoxy-4-iodoamphetamine (DOI) are the most highly studied psychedelics with respect to transcriptional activation, and produce overlapping genetic changes in many parts of the brain, including cortical areas that are known to be important in mediating their effects.

Gene transcription within activated neurons is likely important for the long-term clinical phenomena that are observed following psychedelic administration. For example, the psychological changes that can last months after a single dose of LSD or psilocybin implicate processes that endure over long timescales, as does the existence of hallucinogen-persisting perceptual disorder (HPPD), which can persist for years following a single dose of LSD (Halpern and Pope 2003; Griffiths et al. 2006). Although the mechanisms by which these varied long-term phenomena occur remain obscure, they likely involve changes in long-term synaptic plasticity.

Synaptic events such as late long-term potentiation (late-LTP) require the transcription and translation of a number of genes and proteins, and are thought to be essential in forming memories and mediating learning (Frey et al. 1989; Alberini 2009). Therefore, an understanding of the transcriptional program initiated by psychedelics is necessary to gain insight into the potential long-term clinical benefits and risks of these drugs. Furthermore, examination of the patterns of mRNA transcription produced by psychedelic compounds can provide information about

the signaling pathways these drugs modify acutely, and may offer insight into the mechanisms by which they produce sensory and cognitive changes. For example, the first microarray screen to examine LSD-induced gene expression changes in the prefrontal cortex identified the *ania3* transcript as induced (Nichols and Sanders-Bush 2002). This transcript is from the *homer* locus and encodes for a protein associated with post-synaptic metabotropic glutamate receptor signaling (Kammermeier 2008). Work by others at around the same time demonstrated functional interactions between the 5-$HT_{2A}$ receptor and the mGluR2 receptor, and that metabotropic glutamate receptor signaling is involved in the behaviors produced by psychedelics (Marek et al. 2000).

## 2 Immediate Early Genes

By far, the most well-studied gene expression changes observed following psychedelics administration involve induction of a variety of immediate early genes (IEGs), whose transcription is begun within minutes following neuronal stimulation. IEGs are expressed as a result of a variety of signals that converge on the nucleus and effect transcriptional activators, allowing them to drive subsequent IEG expression. For example, elevations in $Ca^{2+}$ signaling resulting from synaptic activity and membrane depolarization lead to phosphorylation of cAMP response element binding protein (CREB), which binds to specific upstream activating sequences of DNA to induce transcription of certain genes, including *c-Fos*, the prototypical member of a large family of IEGs (Sheng and Greenberg 1990). Fos proteins and other IEGs function primarily as short-lived transcription factors, which themselves initiate a complicated program of further transcription of late-response genes. The specific pattern of gene expression that occurs depends on many factors such as the stimulus, cell type, and second-messenger systems activated. Importantly, the activity-dependent gene expression that begins in the neuronal nucleus ultimately provides a mechanism by which long-term structural and connective changes can occur at the synapse (Kandel 2001; Cohen and Greenberg 2008).

Psychedelics were first shown to induce IEGs in rats through observation of c-Fos protein using immunohistochemistry following the administration of DOI, an agonist that is selective for 5-$HT_2$ receptors. Leslie et al. (1993) demonstrated clear c-Fos-labeling in the amygdala, mamillary nucleus, bed nucleus of the stria terminalis, nucleus accumbens, and cortex following 8 mg/kg DOI. Cortical c-Fos staining was concentrated in the cingulate cortex and in a laminar banding pattern along layer Va in the somatosensory cortex. c-Fos staining appeared nearly exclusively in a subset of neurons labeled with neuron-specific enolase, and never in GFAP-labled astrocytes. DOI-induced c-Fos-labeling was first detected 30 min after treatment and peaked at 3 h, subsequently declining to background levels by 6 h. Further, DOI-induced c-Fos immunostaining was largely eliminated by pretreatment with the 5-$HT_{2A}$ receptor antagonist ritanserin (Leslie et al. 1993). These researchers later described a dose–response relationship concerning c-Fos

expression using escalating doses of DOI ranging from 1 to 32 mg/kg. The density of c-Fos immunoreactivity in the parietal cortex increased over background at 2 mg/kg and reached a plateau at 12 mg/kg (Moorman and Leslie 1998). The authors also noted a high correlation between levels of c-Fos staining and 5-$HT_{2A}$ receptor–ligand binding sites, particularly in layer Va of the cortex (Mengod et al. 1990; Moorman and Leslie 1998). Those early studies clearly indicated that DOI produces a translational signature in neurons that progresses in a dose- and time-dependent manner. They also suggest that this induction follows from 5-$HT_{2A}$ activation in several cortical and sub-cortical regions.

An increase in mRNA levels of IEGs following DOI administration was first reported in the cortex, hippocampus, and cerebellum of rats using northern blot analysis (Tilakaratne and Friedman 1996). The transcription factor genes *c-Fos*, *ngf1c (egr4)*, and *tis1* (*nr4a1*), were increased significantly (219–327%) in the cortex 90 min following 4 mg/kg i.p. DOI. Similar though somewhat smaller responses were observed in the hippocampus and cerebellum in the case of *c-Fos* and *ngf1c*, but large increases in *tis1* expression were restricted to the cortex. All changes were blocked by the 5-$HT_2$ receptor antagonist, ketanserin (Tilakaratne and Friedman 1996). These data confirmed that, in addition to *c-Fos*, other transcription factors (*tis1* and *ngf1c*) are induced by DOI in the cortex, likely through modulation of 5-$HT_2$ receptors. Additionally, this work demonstrated that patterns of DOI-induced gene expression in the brain are regionally specific.

Other types of IEGs that are distinct in function from transcription factors are also induced by psychedelics. Brain-derived neurotrophic factor (BDNF) was the first of these to be observed increasing in response to DOI. BDNF is a widely expressed neurotrophin involved in neuron development, experience-dependent plasticity, and modification of dendritic morphology (Egan et al. 2003; Genoud et al. 2004; Chen et al. 2006). BDNF mRNA was shown with in situ hybridization to increase dose-dependently in the parietal cortex following DOI administration of 0.5 and 2 mg/kg. Interestingly, these same doses caused a decrease below baseline for BDNF expression in the dentate gyrus of the hippocampus. Modulation of BDNF expression by DOI in all regions was abolished using the 5-$HT_2$ receptor antagonist ketanserin and the selective 5-$HT_{2A}$ receptor antagonist MDL100907, indicating that activation of 5-$HT_{2A}$ receptors leads to the increase and decrease of BDNF expression in the cortex and dentate gyrus, respectively (Vaidya et al. 1997). The upregulation of BDNF provides one potential mechanism for psychedelics to modify synaptic strength and connectivity, especially considering the role of BDNF in potentiation of active synapses and ketamine-mediated synaptogenesis (Patterson et al. 1996; Liu et al. 2012).

Further evidence for the widespread initiation of transcriptional activity was supplied when DOI was found to induce *arc* mRNA expression in rats in a dose-dependent fashion (Pei et al. 2000). *Arc* (activity-regulated, cytoskeletal protein) is an effector IEG that displays an affinity for neuronal dendrites, is shuttled to and transcribed at active synapses, and modulates the development of late-LTP and long-term memory (Steward et al. 1998; Guzowski et al. 2000). The expression of *arc* mRNA, examined using in situ hybridization histochemistry, was found to

increase above background after administration of 0.2 mg/kg DOI, with a further increase in magnitude through 2.0 mg/kg in several regions of the cortex, including parietal, frontal, cingulate, and orbital cortex. A slight increase in *arc* mRNA was also noted in the striatum at the highest dose. Consistent with reports for other IEGs, the 5-$HT_2$ receptor antagonist ketanserin completely prevented the increase in *arc* mRNA (Pei et al. 2000).

Arc protein was later shown with immunohistochemistry to follow a pattern of DOI induction similar to the mRNA. At a dose of 1 mg/kg DOI, Arc immunoreactivity was increased robustly throughout the frontal cortex, while slight increases were noted in the caudate putamen. The authors also noted in double-labeling studies that Arc and c-Fos staining were largely overlapping, indicating the same cells were producing these two IEGs (Pei et al. 2004). More recently it was shown that Arc induction following both DOI and stress is reduced upon inducible BDNF loss, suggesting a relationship between the expression of these two genes (Benekareddy et al. 2013). Although playing a role in LTP, Arc also reduces α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA) glutamate receptor signaling (Chowdhury et al. 2006) and contributes to synaptic elimination (Wilkerson et al. 2014). Interestingly, levels of Arc in the cortex are increased after administration of ketamine, which also increases AMPA signaling (Li et al. 2015), demonstrating a complex role for Arc in mediating synaptic plasticity.

## 3 IEG and 5-$HT_{2A}$ Receptor Expression: Early Studies

Several early studies examining the upregulation of IEGs in response to psychedelics made attempts to characterize the cells that were responsive to the drugs. Interestingly, many studies failed to demonstrate c-Fos expression within cells that were labeled with antisera against the 5-$HT_{2A}$ receptor. The first study to report this absence of colocalization administered 8 mg/kg DOI to rats but could find no cells that were co-labeled with antisera against both c-Fos and 5-$HT_{2A}$ receptors (Mackowiak et al. 1999). Other investigators found similar patterns of non-overlapping c-Fos and 5-$HT_{2A}$ receptor staining, and showed that c-$Fos^+$ cells primarily band near the apical dendritic trunk of cells stained with 5-$HT_{2A}$ receptor antibody, but only very rarely found double-labeled pyramidal cells (Scruggs et al. 2000). Using the same receptor antibody as the above two studies, Pei et al. reported similar results showing that DOI-induced Arc protein did not coincide with 5-$HT_{2A}$ receptor antisera reactivity (2004). The dependence of DOI's effects on IEG expression through the 5-$HT_{2A}$ receptor is well documented (Leslie et al. 1993; Tilakaratne and Friedman 1996; Vaidya et al. 1997; Pei et al. 2000; Scruggs et al. 2000), so these observations were initially surprising. However, they should be interpreted with some caution given that more recent studies have indicated psychedelic-induced c-Fos expression occurs almost exclusively in cells that are positive for 5-$HT_{2A}$ mRNA expression (Gonzalez-Maeso et al. 2007). These experiments were performed using fluorescence in situ hybridization (FISH) in both

neuronal cultures and mouse somatosensory cortex following LSD and DOI administration (Gonzalez-Maeso et al. 2007). The disparity between these findings might be explained by differences between mRNA and expression of the 5-$HT_{2A}$ receptor protein, or by the reliance of several of the previous immunohistochemistry studies on one particular antibody to the 5-$HT_{2A}$ receptor that was originally distributed by Pharmingen. Indeed, the pattern of somatodendritic staining produced by the Pharmingen 5-$HT_{2A}$ antibody does not match the staining pattern of other antibodies whose specificity was recently verified in mice lacking the 5-$HT_{2A}$ receptor (Weber and Andrade 2010).

The lack of 5-$HT_{2A}$ immunoreactivity in cells displaying DOI-induced IEG transcription implied that the effect of cellular activation by hallucinogens was indirect. Although the specificity of the antibodies used to make this conclusion is somewhat in doubt, the observations were originally interpreted in the context of early theories positing an important role for thalamocortical afferents in mediating the effects of hallucinogens. This idea was based on a variety of electrophysiological observations in cortical slices, and also explained a reduction of DOI-induced c-Fos expression seen following thalamic lesions (Scruggs et al. 2000; Marek et al. 2001). However, in the light of more recent evidence, cortical–cortical interactions appear critical for hallucinogenic activity. For example, electrolytic thalamic lesions using whole animals rather than slices did not alter cortical responses to DOI, implying that a local cortical mechanism controls neuronal excitability (Puig et al. 2003). Further, a subpopulation of excitatory pyramidal and inhibitory neurons in the cortex can be directly depolarized by 5-$HT_{2A}$ agonists (Beique et al. 2007; Weber and Andrade 2010), and re-introduction of 5-$HT_{2A}$ receptor into primarily cortical areas but not thalamic areas in 5-$HT_{2A}^{-/-}$ knockout mice restores behavioral and transcriptional effects of hallucinogens (Gonzalez-Maeso et al. 2007). These data suggested that IEG induction in vivo may result directly from activation of 5-$HT_{2A}$ receptors on transcriptionally activated cells, through indirect mechanisms (i.e., 5-$HT_{2A}$-mediated glutamate release), or a combination of these events.

## 4 Identification of Tissues that Transcriptionally Respond to Psychedelics: Early Studies

The identification of activated cell populations and the manner by which they initiate gene transcription may provide important information about the mechanisms through which psychedelics cause their effects. Co-labeling studies demonstrated that c-Fos is induced in a variety of cell types in response to psychedelics. For example, 12% of inhibitory GABAergic neurons identified through $GAD_{67}$ labeling in the prefrontal cortex (PFC) also co-stain for c-Fos following 5.0 mg/kg DOI, compared to only 1.4% under control conditions (Abi-Saab et al. 1999). Another unspecified population of cells that did not label for $GAD_{67}$, presumably pyramidal neurons, were also c-$Fos^{+}$ (Abi-Saab et al. 1999). Increases in extracellular GABA following local PFC infusion of DOI also occurs (Abi-Saab et al.

1999). These results are consistent with the idea that, in addition to excitatory neurons, DOI activates inhibitory cells that release GABA in response to stimulation. This activation may be direct because 5-$HT_{2A}$ receptor expression is detected in a population of cortical interneurons, a large fraction of which are parvalbumin positive (de Almeida and Mengod 2007; Weber and Andrade 2010). Furthermore, 5-HT and hallucinogens can depolarize GABAergic interneurons in vitro and in vivo (Foehring et al. 2002; Weber and Andrade 2010; Zhang et al. 2010).

Within the somatosensory cortex, increases in extracellular GABA and glutamate have been observed following systemic DOI, but local infusion of DOI produced increases only in glutamate (Scruggs et al. 2003). Additionally, no GABAergic, c-$Fos^+$ cells were located in the barrel cortex, indicating that a degree of variation exists between the effects of DOI on interneurons across brain regions (Scruggs et al. 2003). Other researchers have noted that DOI-induced c-Fos within the orbital cortex and dorsal medial PFC primarily occurred within $GAD_{67}^+$ cells (Wischhof and Koch 2012). However, little c-Fos and parvalbumin overlap was found in these areas, indicating other interneuron subtypes are activated by DOI (Wischhof and Koch 2012). Although substantial evidence for the activation of neurons has been published, glial cell populations have been largely unstudied with respect to hallucinogens. A single report, however, details co-expression of c-Fos and Olig1, an oligodendrocyte marker, in the PFC following LSD administration (Reissig et al. 2008).

In addition to DOI, other psychedelics produce a variety of genetic responses in the cortex. The first data indicating an IEG response to LSD was published in 1999, and mapped the induction of *c-Fos* mRNA using in situ hybridization. The *c-Fos* staining following 1.0 mg/kg LSD was quite widespread, labeling the frontal and parietal cortex, the striatum, nucleus accumbens, paraventricular nucleus, and a population of cells in the ventral central gray (Erdtmann-Vourliotis et al. 1999). Another report showed similar *c-Fos* mRNA induction, unaltered by morphine pretreatment, which mimicked the laminar pattern seen with DOI-induced *c-Fos* in the cortex (Erdtmann-Vourliotis et al. 2000). Induction of c-Fos protein in the rat brain following 0.5 mg/kg LSD was demonstrated in the mPFC, anterior cingulate cortex, parietal cortex, and in the amygdala (Gresch et al. 2002). The increase in c-Fos immunoreactivity in this study was attenuated by the selective 5-$HT_{2A}$ receptor antagonist M100,907, and was not found in the nucleus accumbens or the ventral striatum (Gresch et al. 2002). It was later demonstrated that a lower, but behaviorally active, dose of LSD (0.16 mg/kg) was able to induce c-Fos in a time-dependent manner in the anterior cingulate cortex, nucleus accumbens shell, paraventricular nucleus, and the bed nucleus of stria terminalis (Frankel and Cunningham 2002). Interestingly, no increases were seen in the prelimbic, frontal, or parietal cortical areas at this dose, nor did LSD increase c-Fos in the nucleus accumbens core (Frankel and Cunningham 2002).

These studies clearly demonstrate the ability of LSD to induce c-Fos expression in many of the same areas where DOI induces expression, with the exception of a less robust response following lower doses of LSD (0.16 mg/kg) in areas of the frontal and parietal cortex (Frankel and Cunningham 2002). Also, expression of IEGs in the nucleus accumbens has been demonstrated with both LSD and DOI

(Leslie et al. 1993; Erdtmann-Vourliotis et al. 2000; Frankel and Cunningham 2002), but is not universally reported with either of these two drugs (Gresch et al. 2002; Wischhof and Koch 2012).

## 5 IEG and 5-HT$_{2A}$ Receptor Expression in the Post-Genomic Era

Widespread surveys of the transcriptional response to psychedelics were made possible with the advent of DNA microarray technology. One of us performed the first unbiased microarray screen on the effects of LSD within the brain and assessed the effects of 1.0 mg/kg LSD on rat PFC 90 min after drug administration. In the first screen, a collection of five genes upregulated by LSD in the PFC were identified: *serum glucocorticoid kinase* (*sgk*), *Iκβ-α*, *neuron derived orphan receptor 1* (*nor1*; *nr4a3*), *ania3*, and *krox-20* (*egr-2*) (Nichols and Sanders-Bush 2002). These genes, along with *Arc* and *c-Fos*, were validated by RNase protection as differentially expressed in the PFC (Nichols and Sanders-Bush 2002).

We subsequently examined time course, dose–response, and sensitivity of the LSD response to 5-HT$_{2A}$ and 5-HT$_{1A}$ receptor antagonists (Nichols et al. 2003). The expression of most of these genes peaked at 90 min and returned to baseline 3–5 h following LSD treatment. The *nor1* gene, however, remained at maximum elevated levels through the final 5 h time point tested (Nichols et al. 2003). Two genes were significantly upregulated at the low dose of 0.20 mg/kg LSD (*krox-20*, *Iκβ-α*), and most expression levels increased with successively higher doses of drug from 0.5 to 1.0 mg/kg. Consistent with reports utilizing DOI, the transcriptional effects of LSD were unaffected by selective antagonism of the 5-HT$_{1A}$ receptor with WAY-100,635 but were significantly attenuated by the 5-HT$_{2A}$ receptor selective antagonist M100,907, with the exception of *sgk* and *Iκβ-α*, which were also unaffected by M100,907. These results indicate that the majority of LSD-related gene expression alterations were induced through activation of the 5-HT$_{2A}$ receptor, but that other receptors contribute to its effects (Nichols et al. 2003).

Extending this work, we performed a second microarray screen using a different Affymetrix gene chip version, and identified and validated three additional transcripts increased by LSD (1.0 mg/kg) in the rat PFC: *map kinase phosphatase 1* (*mkp1*), *core/enhancer binding protein β* (*C/EBP-β*), and the novel gene, *induced by lysergic acid diethylamide 1* (*ilad1*; subsequently renamed *arrestin domain containing* 2, *arrdc2*) (Nichols and Sanders-Bush 2004). Along with the other LSD-induced differentially expressed genes, these also followed a dose- and time-dependent expression pattern. At the highest dose of 1.0 mg/kg LSD, the expression of *mkp1*, *C/EBP-β,* and *ilad* was only partially blocked by M100,907, indicating that activation of multiple receptors is contributing to the effects of LSD on gene expression at this dose (Nichols and Sanders-Bush 2004). Indeed, LSD is a relatively non-selective serotonin (5-HT) and dopamine receptor ligand, with high to moderate affinity for a number of receptors that may contribute to its effects

(Nichols 2004). Interestingly, neither *c-Fos* nor *arc* changes were identified in these two microarray screens. Examination of the raw data suggested equivalent levels between the LSD and control groups. Furthermore, several additional genes were initially called as significantly increased in expression. Each of the genes identified in the primary screen was subject to validation by RNAse protection, where it was found that only 1 in 4–5 genes could be confirmed as differentially expressed. Further, there was no real correlation between expression levels changed between microarray and RNase protection. These results together demonstrate limitations of microarray screens, which are not quantitative, and can result in high false positive and negative rates.

The general functions of the genes induced by LSD are varied, and in the case of some genes mentioned above, little is known. However, a common theme linking the transcriptional changes is an effect on synaptic plasticity. For example, *nor1* is a member of the *Nr4a* family of activity-dependent transcription factors, which has been demonstrated to be important for transcription-dependent LTP in the hippocampus (Bridi and Abel 2013). Similarly, *sgk* has been shown to play a role in long-term memory and the expression of LTP in hippocampal neurons (Ma et al. 2006). *Ania3* is a splice variant within the *Homer1* gene family that encodes synaptic proteins, and has been implicated in mGluR-mediated plasticity (de Bartolomeis et al. 2014; O'Riordan et al. 2014). *C/EBP-β* is known to affect memory consolidation and synaptic strength (Alberini et al. 1994; Taubenfeld et al. 2001), and *Iκβ-α* inhibits NFκB, which is primarily known for its role in inflammatory pathways (Hinz et al. 2012), but is also important in synapse regulation (Salles et al. 2014). However, the manner in which these IEGs contribute to the downstream transcriptional, structural, and functional sequelae of neuronal activation remains poorly understood. Such long-term changes may be important in mediating a variety of behavioral effects of chronic LSD (Marona-Lewicka et al. 2011; Martin et al. 2014).

Transcriptional profiling of cells in culture following the administration of psychotropic agents is one strategy to reveal clues as to the mechanisms by which ligands produces their effects. Gonzalez-Maeso and colleagues initially studied the transcriptional effects of a series of ligands (5-HT, tryptamine, 5-methoxytryptamine, and DOI) in a cell culture system using 5-$HT_{2A}$-expressing HEK293 cells. Although these ligands produced no changes in HEK293 cells without 5-$HT_{2A}$ receptor, they each produced a distinct profile of concentration-dependent gene induction when applied to cells expressing the 5-$HT_{2A}$ receptor. DOI induced the transcription of several genes in this system, including *egr-3*, *egr-2 (krox-20)*, *cox2*, and *cyr61*, whereas 5-HT and tryptamine produced responses of larger magnitude. Both LSD and lisuride (a non-hallucinogenic structural analog of LSD with significantly higher affinity for dopamine $D_2$ receptors), produced very little gene induction in this in vitro system (Gonzalez-Maeso et al. 2003). These researchers further analyzed the effects of DOI on mouse somatosensory cortex using microarray analysis, and validated by RT-QPCR that a subset of genes was differentially regulated. These genes (*c-Fos*, *egr-2*, *N-10*, *Iκβ-α*, *sty kinase*) followed dose- and time-dependent responses, with *sty kinase* being the only downregulated gene (Gonzalez-Maeso et al. 2003). They

also compared the transcriptional effects of LSD, lisuride, and DOI on the expression of 20 genes in mouse somatosensory cortex, which revealed that LSD and DOI upregulated expression of three genes (*egr-1*, *egr-2*, and *period-1*) that were not upregulated by lisuride (Gonzalez-Maeso et al. 2003). A further collection of three genes, including *c-Fos*, was also induced by each ligand in somatosensory cortex. Both LSD and lisuride upregulated *Iκβ-α* in mice lacking the 5-$HT_{2A}$ receptor, however, DOI produced no gene expression changes in these knockout mice (Gonzalez-Maeso et al. 2003), confirming our earlier results of 5-$HT_{2A}$ receptor independent expression of LSD-induced *Iκβ-α*.

These data provide evidence that functional selectivity, a phenomenon whereby different ligands acting through the same receptor can lead to different signaling patterns within the cell (Urban et al. 2007), is occurring at the 5-$HT_{2A}$ receptor. That lisuride induces *c-Fos* expression through the 5-$HT_{2A}$ receptor in primary cultures of mouse cortical neurons (Gonzalez-Maeso et al. 2007), yet does not elicit psychedelics behaviors in humans or produce head-twitch responses in mice, suggests that production of c-Fos is not always correlated with overt behaviors known to be mediated through 5-$HT_{2A}$ receptor activation (Gerber et al. 1985; Gonzalez-Maeso et al. 2003; Halberstadt and Geyer 2013) The two genes, *egr-1* and *egr-2*, which were identified as robustly upregulated by DOI and LSD but not lisuride in somatosensory cortex, represent members of the zinc family of transcription factors whose expression is correlated with LTP and implicated in modulation of synaptic plasticity and memory formation (Richardson et al. 1992; Veyrac et al. 2014). These studies also highlight the difference between in vitro and in vivo models for the study of gene expression, because LSD and lisuride robustly induce gene expression in vivo, but not in vitro in HEK293 cells expressing 5-$HT_{2A}$ receptors (Gonzalez-Maeso et al. 2003).

A subsequent report extended this work to provide more evidence that hallucinogenic and non-hallucinogenic 5-$HT_{2A}$ receptor agonists can produce dissociable effects on transcription and behavior. Hallucinogens (DOI, DOM, DOB, psilocin, mescaline, and LSD) and non-hallucinogens (ergotamine, (*R*)-lisuride, (*S*)-lisuride) were tested in mice expressing the 5-$HT_{2A}$ receptor (*htr2A*$^{+/+}$) and in the receptor knockout mice (*htr2A*$^{-/-}$). Each ligand produced a different transcriptional pattern among the 19 genes tested across several brain regions analyzed including prefrontal and somatosensory cortex, with the overall transcriptional response largely eliminated in the *htr2A*$^{-/-}$ mice. Whereas all ligands tested induced *c-Fos*, only the psychedelic-induced *egr-1* and *egr-2*. *Period-1*, which was specific to psychedelics in the earlier, smaller study, was induced by ergotamine, but not DOM in this larger study, indicating that the transcriptional response to this gene is not psychedelic-specific (Gonzalez-Maeso et al. 2007).

Studies performed in primary neuronal cultures found that whereas both c-Fos and *egr-2* expression were induced by LSD, only c-Fos was increased by R-lisuride (Gonzalez-Maeso et al. 2007). We have examined *egr-2* expression in response to lisuride in several cell types in culture and found that *egr-2* expression by lisuride is dependent on the type of cell used (unpublished data). Therefore, its proposed use as a biomarker for hallucinogenic properties of a drug must be considered with

some degree of caution. Nevertheless, the finding that different 5-$HT_{2A}$ receptor agonists that produce different behaviors can induce differential expression responses in vivo suggests that differential recruitment of signal effector pathways may be important for the behavioral effects of psychedelics.

Interestingly, the application of tetrodotoxin to neuronal cultures, which blocks $Na^+$ channels and action potential firing, had no effect on the ability of LSD to induce transcription of *egr-2* or *c-Fos* (Gonzalez-Maeso et al. 2007). Additionally, these transcripts were present exclusively in cells positive for 5-$HT_{2A}$ mRNA, akin to results seen in layer 5 of somatosensory cortex (Gonzalez-Maeso et al. 2007). These data demonstrate that, at least in cell culture, activation of 5-$HT_{2A}$ receptors can directly alter gene expression without a requirement for neuronal depolarization. Taken together, the cell culture and brain tissue data indicate that the transcriptional response mediated by 5-$HT_{2A}$ receptor activation is dependent on the ligand, the cell type, and the environment in which the receptor resides, factors that vary considerably between neuronal cell culture and the brain.

## 6 Psychedelics, Gene Expression, and Cellular Signaling

The wide range of effector pathways recruited by the 5-$HT_{2A}$ receptor generates a complex signaling network. Although many effectors are presently known, there are likely many more that remain to be discovered. The canonical signaling pathway involves positive coupling to $G\alpha_q$, which activates PLC-β, leading to phosphatidylinositol (PI) hydrolysis, the release of intracellular calcium, and the activation of protein kinase C (PKC). The 5-$HT_{2A}$ receptor can also couple to other G proteins and downstream effector pathways. For example, receptor stimulation can activate phospholipase $A_2$ ($PLA_2$) and the production of arachidonic acid (AA) independently of $G\alpha_q$ and PLC-β (Felder et al. 1990; Berg et al. 1998; Kurrasch-Orbaugh et al. 2003b). The activation of $PLA_2$ through the 5-$HT_{2A}$ receptor is complex, and can involve several pathways that include a $G\alpha_{i/o}$-associated $G\beta\gamma$ pathway through Src, and $G\alpha_{12/13}$ activation of Rho (Kurrasch-Orbaugh et al. 2003a). Interestingly, the interoceptive behavioral cues elicited by psychedelics in drug discrimination assays in rodents correlate with activation of the $PLA_2$ pathway through $G\alpha_{i/o}$ rather than the PLC-β pathway through $G\alpha_q$ (Kurrasch-Orbaugh et al. 2003a). Additional pathways linked to 5-$HT_{2A}$ receptor stimulation include pERK activation through β-arrestin (Schmid and Bohn 2010), and phospholipase D (PLD) activation through the small G-protein ADP-ribosylation factor (ARF) (Barclay et al. 2011). Each of these pathways could conceivably recruit expression of different sets of genes such that, depending on the ligand used to activate the receptor and the nature of the cell it is expressed in, gene responses could be vastly different.

Further exploration of gene expression differences between LSD and lisuride in neuronal cell culture has found that inhibition of PLC-β with U73122 eliminates the transcriptional response to both LSD and (*R*)-lisuride, but that pertusis toxin

(PTX) only lowers the magnitude of transcriptional response to LSD (*c-Fos*, *egr-1*, *egr-2*) and not (*R*)-lisuride (*c-Fos*) (Gonzalez-Maeso et al. 2007). The Src inhibitor PP2 prevents LSD from inducing *egr-1* and *egr-2*, but allows for equivalent *c-Fos* induction between LSD and (*R*)-lisuride (Gonzalez-Maeso et al. 2007). These results suggest that both $G\alpha_q$ activation of PLC-β and activation of $G\alpha_{i/o}/G\beta\gamma$/Src is necessary for psychedelic relevant gene expression patterns, consistent with previous pharmacological data (Kurrasch-Orbaugh et al. 2003a; Gonzalez-Maeso et al. 2007). These findings do not, however, preclude the involvement or necessity of additional signaling pathways in the genetic response to psychedelics.

Several studies have attempted to modify the behavioral response to psychedelics by disrupting signaling pathways downstream of 5-$HT_{2A}$ receptor stimulation. For example, dexamethasone, a glucocorticoid that inhibits $PLA_2$, and indomethacin, which prevents the conversion of AA into other signaling molecules through the inhibition of COX enzymes, were used in combination with DOI. Both indomethacin and dexamethasone each reduced c-Fos expression in the cortex by ~50%, but did not eliminate it (Mackowiak et al. 2002). In mice lacking the $G\alpha_q$ protein, c-Fos induction following DOI is abolished, and the head-twitch response is markedly reduced (Garcia et al. 2007). Further, PLC-β activation is necessary for the head-bob response produced by intra-cortical DOI in rabbits, although LSD-induced head bobs were unaffected by PLC-β inhibition (Schindler et al. 2013). These studies provide further evidence that multiple pathways downstream of receptor activation are important for transcriptional and behavioral effects of psychedelics in vivo.

IEG expression has frequently been used as an output to measure perturbations of psychedelic drug mediated signaling. For example, no increase in c-Fos immunoreactivity was observed in somatosensory cortex when an AMPA receptor antagonist, GYKI 52466, preceded DOI administration (Scruggs et al. 2000). In a separate study, GYKI 52466 (25 mg/kg, i.p.) attenuated the increase in Arc protein seen following DOI in all cortical areas examined (Pei et al. 2004). The NMDA antagonist MK-801 blocked the increase of Arc in frontal, orbital, and cingulate cortex, but not in parietal cortex where MK-801 given alone induces Arc expression (Pei et al. 2004). These results suggest the importance of AMPA and NMDA receptor activation for the induction of Arc expression, and by extension implicate glutamate transmission as an essential element in DOI's transcriptional effects. Further supporting this idea, double immunofluorescence for mGluR2/3 receptors and NMDAR1 receptors revealed the great majority of c-$Fos^+$ cells are positive for these AMPA and NMDA subunits. These data indicate that ionotropic glutamate receptor activation is necessary for neurons to be transcriptionally activated by DOI (Pei et al. 2004). These results are consistent with a preponderance of evidence implicating glutamate release as critical for the electrophysiological and behavioral responses to hallucinogens (Aghajanian and Marek 2000; Scruggs et al. 2003; Muschamp et al. 2004).

The characterization of metabotropic glutamate receptor 2 (mGluR2) influences on 5-$HT_{2A}$ signaling has also relied partly on measurement of IEG expression. For example, pretreatment of rats with the mGluR2/3 agonist LY35470

dose-dependently and completely (at 10 mg/kg) prevented the upregulation of *BDNF* mRNA in the medial prefrontal cortex (mPFC) caused by 5 mg/kg DOI (Gewirtz et al. 2002). This blockade extended to other frontoparietal regions of the cortex and to the claustrum, however, LY35470 did not prevent the DOI-mediated upregulation of *BDNF* in the intralaminar and midline thalamic nuclei. Consistent with these data, the mGlurR2/3 antagonist LY341495 significantly potentiated the upregulation of *BDNF* by 5 mg/kg DOI (Gewirtz et al. 2002). These results are consistent with electrophysiological and behavioral data showing that mGlurR2/3 receptor activity attenuates the effects of psychedelics acting at the 5-$HT_{2A}$ receptor, and that 5-$HT_{2A}$ and mGluR2/3 receptors are localized to similar structures in the mPFC (Gewirtz and Marek 2000; Marek et al. 2000). With respect to IEG expression, activation of mGlurR2/3 with LY379268 attenuates DOI-induced *c-Fos* expression in the mPFC, but not the frontoparietal or somatosensory cortex (Zhai et al. 2003). Because the positive allosteric modulator of the mGluR2 receptor, biphenyl-indanone A (BINA), reduces (*R*)-DOB-induced c-Fos expression in the mPFC, but not somatosensory cortex, it is likely that mGluR2 and not mGluR3 receptors are involved in psychedelic-induced c-Fos expression changes (Benneyworth et al. 2007). These data together indicate that there is a strong functional relationship between 5-$HT_{2A}$ and mGluR2 receptors with respect to IEG expression, at least within the mPFC. This relationship extends to other aspects of psychedelic-induced effects because mGlurR2 activation also reduces psychedelic-induced EPSCs and behavioral head-twitch responses. The mechanism for the functional interaction between 5-$HT_{2A}$ and mGluR2 signaling is not completely understood. Although there are reports of heterodimerization between these two receptors (Gonzalez-Maeso et al. 2008; Moreno et al. 2011), this conclusion has been controversial (Delille et al. 2013). Interestingly, in support of functional heterodimerization between these two receptors in the mechanism of action of psychedelics, presynaptic 5-$HT_{2A}$ receptors have recently been identified on thalamic inputs in the cortex (Barre et al. 2016).

## 7 Identification and Characterization of the Cortical Cellular Population Responsive to Psychedelics

Although the importance of 5-$HT_{2A}$ receptor signaling in the cortex for the transcriptional and behavioral effects of psychedelics has long been appreciated, the precise population of cells responsive to psychedelics that initiates the signaling that leads to psychedelic transcriptional and behavioral effects remains has only recently been studied. Experiments using Cre recombinase under control of the *Emx1* promoter to restore 5-$HT_{2A}$ receptor expression in cortical pyramidal cells of *htr2A*$^{-/-}$ mice (Gorski et al. 2002) revealed that 5-$HT_{2A}$ receptor signaling in these neurons is sufficient to recapitulate the transcriptional (*c-Fos*, *egr-1*, *egr-2*) response to LSD, along with the behavioral head-twitch responses to LSD and DOI

(Gonzalez-Maeso et al. 2007). Therefore, 5-$HT_{2A}$ receptor signaling within the *Emx1* lineage, including glutamatergic neurons and glia in the cortex, but not GABAergic interneurons or non-cortical neurons, is necessary and sufficient for at least some of the effects of psychedelics.

Recently, we optimized neurocytometry methodology to isolate and analyze populations of cells within the brain that transcriptionally respond to psychedelics (Martin and Nichols 2016; Martin et al. 2017). Somewhat surprisingly we found that only ~5% of cortical neurons directly respond to psychedelics in vivo by increasing transcription of immediate early and other genes. These genes include those for *c-Fos*, Δ*fosB*, *krox20/erg2, and per1* (Martin and Nichols 2016). So far, the only feature found to distinguish these neurons from those that do not respond transcriptionally has been that the responding cells have a significantly higher level of *HTR2A* mRNA expression, which may result in higher 5-$HT_{2A}$ receptor levels rendering the neurons more sensitive to agonists for this receptor. We speculate that activation of this 5% of cortical neurons, which we have termed the 'trigger population', is necessary to initiate the cascade of events leading to changes in the default mode network and behavioral alterations, and may be the same small population that was earlier identified by electrophysiological experiments to depolarize in the presence of 5-$HT_{2A}$ receptor agonists (Beique et al. 2007). In addition to excitatory cortical neurons, we found that ~5 to 10% of inhibitory GABA neurons are transcriptionally activated by psychedelics. Because these activated interneurons, which are comprised of only the somatostatin and parvalbumin subclasses, do not express higher levels of the 5-$HT_{2A}$ receptor than non-transcriptionally activated interneurons, we believe that their activation is primarily indirect (Martin and Nichols 2016). Interestingly, small populations of non-neuronal cells like astrocytes also become transcriptionally active for genes like *c-Fos* following administration of psychedelic drugs (Martin and Nichols 2016). We also found that transcriptional responses differed between brain regions analyzed. For example, somatostatin interneurons are transcriptionally activated in somatosensory cortex, but not medial prefrontal cortex, and *mGluR2* expression in general was higher in responding populations of neurons compared to non-responding neurons in somatosensory cortex compared to medial prefrontal cortex (Martin and Nichols 2016).

## 8 Chronic Effects of Psychedelics

In addition to producing acute molecular and behavioral effects, LSD also produces long-lasting changes in gene expression and behavior when given chronically. We initially reported that rats given 0.16 mg/kg LSD every other day for 90 days exhibit a variety of behavioral alterations, including hyperactivity in an open-field, reduced sucrose preference, and changes of social behaviors (Marona-Lewicka et al. 2011). Interestingly, some of these behaviors, such as increased locomotion, are persistent at full strength long after the drug is discontinued (Marona-Lewicka et al. 2011), indicating that long-term LSD administration in rats may permanently

shift brain neurochemistry and gene expression from a normal to a pathological state. These altered phenotypes represent several domains of the Research Domain Criteria matrix (Morris and Cuthbert 2012), including negative and positive valence systems, and social processes.

To investigate how long-term LSD administration affects gene expression in the brain, we performed RNA sequencing on RNA isolated from the mPFC of rats four weeks after cessation of a 90-day treatment protocol with LSD or saline (Martin et al. 2014). We found several hundred relatively low-magnitude (two-fold) yet significant transcriptional changes in the mPFC of LSD-treated animals long after drug administration stopped. Functional clustering analysis indicated that the altered genes were significantly concentrated in pathways related to neurotransmission, synaptic plasticity, and metabolism (Martin et al. 2014). Several unanticipated clusters of genes were identified that included those involved in RNA processing and endocrine function (Martin et al. 2014). We also found a significant enrichment for altered transcripts whose homologs in humans have been implicated in schizophrenia by others. These include genes for the dopamine $D_1$ and $D_2$ receptors, BDNF, ERBB4, and various NMDA and GABA receptor subunits (Martin et al. 2014).

Persistent connectivity modifications produced by long-term LSD are likely mediated through general plasticity mechanisms that begin with the sustained activation of neuronal ensembles and resultant changes in transcription and translation that alter synaptic function (Leslie and Nedivi 2011). The wave of genes induced by psychedelic drug administration functions partially to initiate a stereotyped cascade of complex late-response transcription that can nevertheless alter neuronal function and connectivity in a highly coordinated fashion (Lyons and West 2011). Our early microarray studies demonstrated that that acute administration of LSD induces a small collection of immediate early genes and transcription factors. Although most of these return to baseline expression within several hours, some do not. We hypothesize that with repeated LSD administration, the genes that remain differentially expressed serve to both subtly alter cellular function and recruit additional genes to a dysregulated state. After a certain window of time, between 6 and 12 weeks of treatment, the cellular changes reach a critical and self-sustaining point such that when drug administration ceases the brain has shifted to an abnormal state. Because of the nature of the abnormal behaviors produced and the genes that are affected, we have proposed that rats treated with LSD for three months may serve as a useful platform to study mechanisms underlying behaviors relevant to certain psychiatric diseases (Martin et al. 2014).

## 9 Effects of Psychedelics Outside of the CNS

Outside of the CNS, there has been little study of the effects of psychedelics on gene expression. That may be because psychedelics are primarily thought of as CNS active agents devoid of effects in the periphery. The 5-$HT_{2A}$ receptor is, however, the most widely expressed serotonin receptor in the mammalian body and

found to be expressed in nearly every tissue and cell type. Psychedelics would therefore be predicted to have effects on these peripheral tissues, including effects on gene expression. We have investigated the role of psychedelics in the periphery, and have discovered them to be powerful anti-inflammatory agents. At extremely low doses, drugs like (*R*)-DOI and LSD can inhibit inflammation mediated by the proinflammatory agent tumor necrosis factor alpha (TNF-α) in both cell culture and in whole animal (Yu et al. 2008; Nau et al. 2013). When administered directly to the lung through nebulization, (*R*)-DOI potently prevents the development of allergic asthma and associated inflammation in a mouse model (Nau et al. 2014). The effects of 5-$HT_{2A}$ receptor activation by (*R*)-DOI on gene expression in peripheral tissues (e.g., vascular, gut, lung) are consistent, and include a reduction in mRNA levels of several inflammatory related cytokines and chemokines such as *Il-6*, *Il-5*, *Il-1b*, *Il-13*, *GMCSF*, and *Mcp1* (Nau et al. 2013, 2014). Although the precise mechanism for inhibition of transcription of proinflammatory genes remains to be elucidated, we believe that stimulation of 5-$HT_{2A}$ receptors with psychedelics acts through specific isoforms of PKC to inhibit signaling from the TNF-α receptor, and inhibits activation of NF-κB. Interestingly, our earlier microarray studies found that LSD increases expression of the gene encoding for IκB, the main inhibitory protein of NF-κB, in the brain (Nichols et al. 2003). There are no reports in the literature, however, examining the effects of psychedelics on neuroinflammation and associated gene expression and any potential effects remain to be fully elucidated.

## 10 Conclusion

Clinical studies on psychedelic compounds conducted through the early 1970s explored a variety of potential uses for psychedelics, including the treatment of various mental disorders and addictions (Baker 1964; Savage and McCabe 1973; Krebs and Johansen 2012). Renewed clinical interest in these drugs has followed along this path in recent years, and a small group of studies has been performed using psilocybin as an anxiolytic/antidepressant in terminal cancer patients, as a treatment for obsessive compulsive disorder, and as a treatment for nicotine addiction (Moreno et al. 2006; Grob et al. 2011; Johnson et al. 2014). LSD also has recently been tested as an adjunct to psychotherapy in terminal illness (Gasser et al. 2014). Our recent work with (*R*)-DOI may lead to clinical therapies for inflammatory disorders like asthma (Nau et al. 2014).

Generally, psychedelics have been recognized for their ability to occasion mystical-type experiences. In one study, a single administration of psilocybin to healthy humans had a positive effect on mood and well-being that persisted for at least 14 months (Griffiths et al. 2006, 2011), and there have been several recent publications describing the efficacy of one or two treatments with psilocybin to long-lasting antidepressant effects and treat addiction (Griffiths et al. 2016; Johnson et al. 2017). In patients, it is reasonable to speculate that a single administration of a psychedelic may be producing long-lasting positive behavioral and/or physiological

changes through long-term alterations in gene expression. In the event that larger clinical studies can further establish therapeutic value for psychedelics, it will be very exciting to elucidate which changes in gene expression are ultimately responsible for their clinical efficacy.

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