Neuronal Genes Affected by LSD Microdosing after Cessation

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Introduction

Microdosing is the ingestion of psychedelic compounds in minute sub-hallucinogenic doses over longer periods of time, unlike the traditional single, large dose. According to media, microdosing with Lysergic acid diethylamide (LSD) or psilocybin mushrooms has gained substantial popularity in non-clinically supervised psychedelic users, otherwise a relatively young demographic (age_{μ} = 26.9 years, standard deviation = 8.6) that may not have yet achieved full neurological development (Rosenbaum *et al.*, 2020). The common appeal for microdosing stems from unbacked claims of improved cognition, creativity, focus, and decreased symptoms of depression and anxiety (Anderson, 2019; Polito, 2019). Furthermore, the longterm neuromodulatory effects of LSD microdosing are still unknown in terms of safety and evidence-based effects. Understanding the biomolecular changes associated with microdosing is essential for evidence-based clinical practice if psychedelic class drugs, like those of MindMed®, become pharmaceutical products.

It is known that concurrent activation of multiple neuromodulatory systems with psychedelics can induce long-lasting changes in our neurobiology (Calvey, 2018). This is unique since very few pharmacological agents can elicit long-lasting effects after a single administration. A rare example might be Botox, but most drugs are used for short-term administration (antibiotics, caffeine…) to chronic administration (insulin, beta blockers, statins…). Psychedelics, after a single exposure, may induce neuromodulatory effects on gene expression that persist long beyond pharmacokinetic elimination (Nichols, 2002). Various studies on patients treated with one to two carefully guided, high-dose psychotherapy sessions showed significantly decreased symptoms of anxiety (after 12 months), post-traumatic stress disorder (after 12 months), and treatment-resistant depression (after 5 weeks and up to 6 months), albeit with variable effect sizes (Gasser, 2015; Gasser, 2014; Mithoefer, 2018; Carhart-Harris, 2017; Mccorvy, 2016). How can improvements in symptoms remain months after the drug has been metabolized from the body?

In the case of a serotonin receptor, the 5-HydroxyTryptamine$_{2A}$ receptor, its abnormal expression in the amygdala is associated to depression, addiction, anxiety, post-traumatic stress disorder, and suicidal behaviour (Carhart-Harris, 2017; Mccorvy, 2016; Bogenschutz & Johnson, 2016; Pandey, 2002). Yet, when a potent 5-HT_{2A} agonist such as LSD is administered, longterm 5-HT_{2A} downregulation may help to physiologically explain longterm symptom alleviation (Oram,

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2018; Mccorvy *et al.*,2016). Therefore, lasting effects on psychological status ought to reflect lasting changes in neurobiological gene expression (GE).

To investigate the longterm impacts of LSD microdosing, we suppose that any perceived psychological impact reflects measurable changes in neurobiological GE. Thus, it's hypothesized that prolonged LSD microdosing can modulate long-term neuronal GE in humans, even after many years of cessation. This study uses rodents exposed to LSD microdoses and performs a hypothetical test on each neuronal gene at the start of cessation, and many years afterwards, to evaluate exactly which genes have altered states of expression and if these changes are permanent. Therefore, results from this study identify the genes whose expression is affected by LSD microdosing via a suitable animal model for humans, the *Rattus norvegicus*.

Experimental Approach

For the purpose of this study, human brain development can be divided into five categories: early development (0 – 17 years), late development (18 – 24 yrs), peak functioning (25 – 30 yrs), neurological senescence (31 – 60 yrs), and accelerated degeneration (61+ yrs – but is largely variable). Neurological Maturity (NM) is defined as the years from peak functioning to the end of neurological senescence (25 – 60 yrs). Since tests will be performed on rodents, human to rodent age will be translated appropriately (Fig.1, Appendix). Further, rodents should make for excellent animal models since to humans they show somewhat similar brain distributions of 5-HT receptors – a high affinity target of LSD (Pazos, 1985; 1987).

The experiment exposes one group of rodents to LSD microdosing throughout late development (Group 1), and another group throughout NM (Group 2). Group 2 controls for the effects of NM on GE. A third group will serve as a negative control, receiving no LSD (Group 0). Neuronal GE is measured using RNA-sequencing at the start of NM; the cessation point for group 1 (25 years in humans); and at the end of NM (60 years in humans) before the potential for accelerated degeneration confounds values for GE. To reiterate, group 1 who is exposed to LSD microdosing during late development (18 – 25 yrs) serves to measure gene modulatory effects at cessation and many years after cessation at the end of NM. See the Appendix for an experimental visualization.

RNA-seq can measure genome wide GE in any given neuron by quantifying the mRNA transcriptome. It's particularly useful for two reasons: transcript abundance is directly proportional

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to GE (a higher mRNA count suggests a more transcribed and active gene) and data analysis is more valuable for organisms whose genome has already been sequenced (Mortazavi, 2008; Cloonan, 2008). However, this technique is not perfect and can be confounded by sequence bias (under/over counting of particular gene fragments), and post-transcriptional controls of GE which silent gene products despite high transcript abundance (Liu, 2016). Neurons will be extracted using the method of Zhang (2014) that incorporates immunopanning to sort and isolate neurons from extracted tissue, total RNA isolation via Qiagen miRNeasy kits, mRNA purification via poly-A selection, cDNA generation via reverse transcription of mRNA, and sequencing of that cDNA library via Illumina HiSeq 2000. The sequencing read data for rodents will be downloaded from the Gene Expression Omnibus.

We can control for the variation of GE across different brain tissues by sampling neurons from three regions: the neocortex, the amygdala, and the nucleus accumbens. These were chosen based on their pathophysiological significance in human cases of degenerative disease, anxiety, depression, epilepsy, and addiction. Variation in GE across developmental phases are accounted for with two experimental groups exposed during different phases of development and by measuring GE at the start and end of NM. The confounding neurological effect of isolation is accounted for by supporting social cohesion between rodents of an experimental group. Stress on administration is avoided since LSD is supplemented in their dietary intake. Microdoses in humans average at 13.5 μg (or 0.22 μg/kg) every two days (Polito and Stevenson, 2019), so adjusting for rodent body weights, 17ng – 55ng of LSD will be diluted over a 2 day dietary intake. Finally, post-transcriptional controls of GE and cross-specie LSD target sites cannot practically be controlled for unless only a few select genes are assessed.

At the start of NM, 20 rodents from Groups 0 and 1 will be sacrificed (n = 20), and at the end of NM, 20 more rodents from Groups 0, 1, and 2 will sacrificed (Fig.1, Appendix). GE across each brain tissue (amygdala, neocortex, nucleus accumbens) will be averaged across 20 samples from each group. Since Groups 0 and 1 are sampled twice, their initial population numbers will be 40. We are assuming that the variances in GE between each group is similar and that the expression pattern of a given gene is normally distributed. Since there is only one dependent (mRNA transcript count) and one independent variable (period of LSD exposure) for every gene, comparisons will be evaluated using one way ANOVA. Since there are roughly 22’250 coding genes to test (Rat Genome Sequencing Project Consortium, 2004) and 15 comparisons per gene (Fig.2, Appendix),

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there will be a total of 333’750 comparisons. Thus, we can expect a significance cut-off point, p-value $= \frac{0.05}{333\,750} \approx 1.5\text{x}10^{-7}$. Since the standard deviation for each gene’s expression is unique, a simple mathematical rule for significance of effect size can be written. Based on a sample size of 20, a p-value of $1.5\text{x}10^{-7}$, and a statistical power of 90%, only effect sizes (changes in mean mRNA counts) that are greater than 2.07x the respective SD will return significant. For example, if the mRNA count is 1000 with a SD of 100, the comparison must have a mean mRNA count of at least 1207 (+2.07 * 100) or at most 793 (-2.07 * 100) to be considered significant.

Implications

With a recent rise in unprescribed and unmeasured LSD microdosing trends, understanding how LSD affects our neuronal GE over long periods of time should be reported. Identification of the affected neuromodulatory systems can better support hypotheses for true changes in neurochemistry. This includes changes in neurotransmitter synthesis, the production of brain-derived neurotrophic factor, and the regulation of receptors involved in impulsivity and the pathophysiology of depression, anxiety, and other psychologically destabilizing conditions. The fact that the benefits and risks of LSD microdosing are backed only by anecdotal claims carries no scientific validity. Knowing that users start self-administration with the expectation of positive outcome, placebo effects and true neurobiological effects must be distinguished. Recognizing that psychedelic compounds have the power to leave lasting neurobiological imprints; whether beneficial or harmful; confirming their existence and identifying them is the objective of this study, which should offer a strong inference on human neurobiology despite the use of a rodent model.

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Appendix: Visualizing Experimental Theory

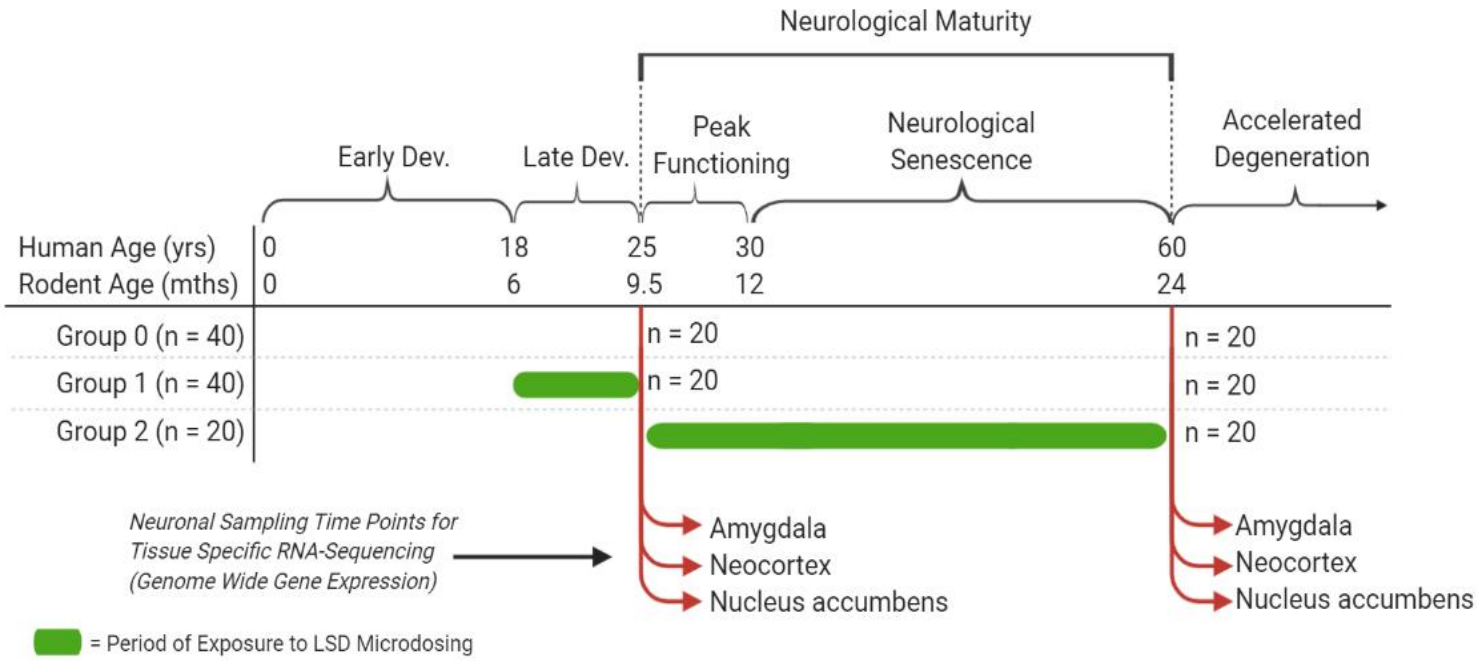

Figure 1. A visual representation of experiment structure for Groups 0 – 2 and their frame of exposure to LSD microdosing across five phases of brain development. Human to Rodent ages are translated from (Sengupta, 2013) and 20 samples are sacrificed from each experimental group at the start and end of Neurological Maturity (NM). Neurons are sampled tissue specifically from the amygdala, neocortex, and nucleus accumbens. Years (yrs); Months (mths).

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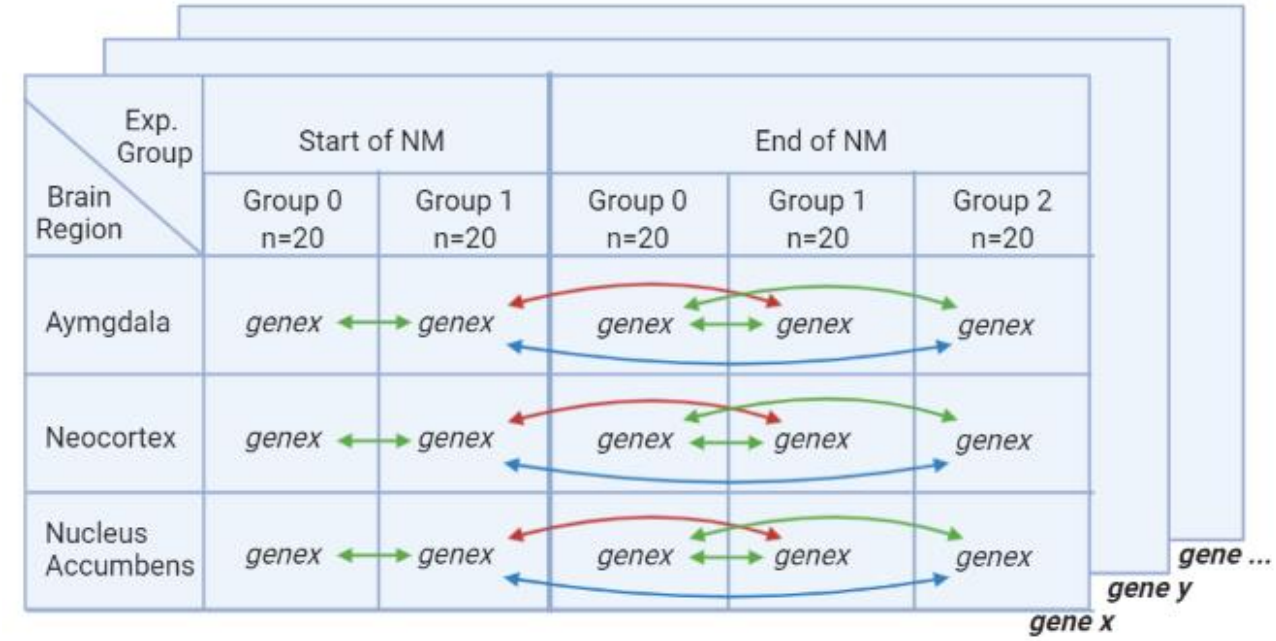

Figure 2. A visual representation of the statistical comparisons and what they're used for. **Green**: tests for any significant difference at cessation relative to negative controls. **Red**: tests for any significant changes in GE many years after the cessation of LSD microdosing. **Blue**: tests if exposure before Neurological Maturity or during Neurological Maturity had effects on GE. Neurological Maturity (NM). Comparisons per gene = 15.

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