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# Pharmacological characterization of the LSD analog *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA)

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**Keywords:** LSD; 5-HT<sub>2A</sub> receptor; lysergamide; psychedelics; head twitch

## ABSTRACT

*Rationale:* The lysergamide lysergic acid diethylamide (LSD) is a prototypical classical hallucinogen with remarkably high potency. LSD remains a popular recreational drug but is also becoming an important research tool for medical and neuroscience studies. Recently, several lysergamides that are close structural analogs of LSD have been sold as recreational drugs, which suggests that further studies are needed to explore the pharmacological properties of these compounds. *Objective:* In this present investigation, another LSD congener, *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA), which to date has not been marketed as a recreational substance, was evaluated for its pharmacological features relative to those previously reported for LSD. The experiments focused on interactions with the 5-HT<sub>2A</sub> receptor, which is responsible for mediating the psychedelic effects of LSD and other hallucinogens. *Methods:* Competitive binding assays were performed to measure the affinity of ECPLA for 27 monoamine receptors. The ability of ECPLA to activate human 5-HT<sub>2</sub> receptor subtypes was assessed using calcium mobilization assays. Head twitch response (HTR) studies were conducted in C57BL/6J mice to determine whether ECPLA activates 5-HT<sub>2A</sub> receptors *in vivo*. Two other *N*-alkyl substituted lysergamides, *N*-methyl-*N*-isopropyl lysergamide (MIPLA) and *N*-methyl-*N*-propyl lysergamide (LAMPA), were also tested in the HTR paradigm for comparative purposes. *Results:* ECPLA has high affinity for most serotonin receptors,  $\alpha_2$ -adrenoceptors, and D<sub>2</sub>-like dopamine receptors. Additionally, ECPLA was found to be a potent, highly efficacious 5-HT<sub>2A</sub> agonist for Gq-mediated calcium flux. Treatment with ECPLA induced head twitches in mice with a median effective dose (ED<sub>50</sub>) of 317.2 nmol/kg (IP), which is ~40% of the potency observed previously for LSD. LAMPA (ED<sub>50</sub> = 358.3 nmol/kg) was virtually equipotent with ECPLA in the HTR

paradigm whereas MIPLA ( $ED_{50} = 421.7$  nmol/kg) was slightly less potent than ECPLA.

*Conclusions:* These findings demonstrate that the pharmacological properties of ECPLA, MIPLA and LAMPA are reminiscent of LSD and other lysergamide hallucinogens.

## INTRODUCTION

(+)-Lysergic acid diethylamide (LSD; Figure 1) is one of the most potent serotonergic hallucinogens, with typical oral doses ranging from 60–200 µg (Shulgin and Shulgin 1997). The activity of LSD is closely tied to the specific structure of its diethylamide moiety, and even minor modifications produce a dramatic reduction of potency. Replacement of the diethylamide group of LSD with another alkylamide group or a cycloalkylamide moiety generally reduces hallucinogenic potency by at least an order of magnitude (Rothlin 1957; Murphree et al. 1958; Abramson 1959; Isbell et al. 1959; Abramson and Rolo 1967). Even *N*-methyl-*N*-propyl lysergamide (LAMPA, MPLA or LMP-55), an isomer of LSD with similar hydrophobicity, is markedly less potent (Abramson and Rolo 1967). One exception is the conformationally restricted LSD analog (2'*S*,4'*S*)-lysergic acid 2,4-dimethylazetidide (LSZ), which retains high 5-HT<sub>2A</sub> affinity and potency in rodent behavioral models (Nichols et al. 2002; Brandt et al. 2017a).

Although very few clinical investigations were carried out with LSD during the past few decades, numerous studies have been published in recent years. Some of these investigations were conducted to assess potential therapeutic applications (Gasser et al. 2014,2015), but most studies were intended to assess the subjective response to LSD (Schmid et al. 2015; Carhart-Harris et al. 2016a; Dolder et al. 2015,2016; Schmid and Liechti 2017) or to evaluate its neurophysiological effects (Carhart-Harris et al. 2016b; Tagliazucchi et al. 2016; Müller et al. 2017a,b; Schmidt et al. 2017). Thus, LSD is rapidly becoming an important tool in medical research.

There has been considerable interest in the pharmacology of LSD due to its high potency. LSD has been found to have high affinity for most serotonin (5-hydroxytryptamine, 5-HT)

receptors and also interacts with dopaminergic and adrenergic receptors (Leysen 1989; Peroutka 1994; Marona-Lewicka and Nichols 1995; Watts et al. 1995; Nichols et al. 2002; Rickli et al. 2015). The psychedelic effects of serotonergic hallucinogens are largely mediated by 5-HT<sub>2A</sub> receptor activation; blockade of the 5-HT<sub>2A</sub> receptor eliminates the subjective effects produced by LSD and other hallucinogens in humans (Vollenweider et al. 1998; Valle et al. 2016; Barrett et al. 2017; Kraehenmann et al. 2017; Preller et al. 2017). A recent x-ray crystallography study of LSD bound into the 5-HT<sub>2B</sub> receptor, along with receptor kinetics studies and molecular dynamics simulations, revealed that extracellular loop 2, which connects the top of helix 4 with helix 5, folds over the LSD molecule after it binds, dramatically hindering its dissociation from the receptor (Wacker et al. 2017). This “entrapment” of LSD within the receptor may at least partially account for the high potency and long duration of action of this molecule. Nevertheless, pharmacokinetic studies in humans indicate that the subjective effects of LSD occur in parallel with its presence in the body at effective concentrations (Dolder et al. 2015,2017).

LSD has been a popular recreational drug since the mid-1960s. Although the use of LSD has occurred at relatively stable levels, in recent years several other lysergamides have been distributed as new psychoactive substances (NPS) or “research chemicals” (Brandt et al. 2016,2017a,b,2018). Most of the new lysergamides, including *N*<sup>6</sup>-allyl-6-norlysergic acid diethylamide (AL-LAD), *N*<sup>6</sup>-ethyl-6-norlysergic acid diethylamide (ETH-LAD), LSZ, and lysergic acid morpholide (LSM-775), were originally synthesized during the course of scientific research (Gogerty and Dille 1957; Hoffman and Nichols 1985; Nichols et al. 2002). Other compounds, however, were specifically developed by NPS vendors as potential recreational drugs. The lysergamides 1-propionyl-LSD (1P-LSD) and 1-propionyl-ETH-LAD (1P-ETH-

LAD) were previously unknown in the scientific literature before they were marketed online as psychoactive substances (Brandt et al. 2016,2017b).

According to recent online postings, NPS vendors claimed to have synthesized *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA; Figure 1), a novel lysergamide that is unknown in the scientific literature. Therefore, we have compared the pharmacological properties of ECPLA to those previously reported for LSD. Competitive binding assays were performed to measure the affinity of ECPLA for 27 monoamine receptors. Additionally, activation of 5-HT<sub>2</sub> receptor subtypes by ECPLA was assessed using functional assays of Gq-coupled Ca<sup>2+</sup>-mobilization.

In addition to assessing whether ECPLA is likely to become a new recreational drug, another reason to investigate its pharmacology is to probe the structure-activity relationships governing the interactions between lysergamides and the 5-HT<sub>2A</sub> receptor. Specifically, the studies were designed to test whether replacing one of the ethyl groups of LSD with a cyclopropyl substituent has a significant effect on 5-HT<sub>2A</sub> receptor affinity and potency. The potency of LSD is significantly reduced by increasing the steric bulk of one of its amide substituents, as exemplified by *N*-ethyl-*N*-isopropyl lysergamide (EIPLA), which had three-fold lower affinity than LSD for 5-HT<sub>2A</sub> sites labeled with [<sup>3</sup>H]ketanserin in rat frontal cortex and almost 3-fold lower potency than LSD in the rat drug discrimination paradigm (Huang et al. 1994). However, it is not clear how potency would be affected by a marginal increase in steric bulk. The steric bulk of a cyclopropyl group (with a calculated van der Waals volume of 43.8 Å<sup>3</sup>) is more comparable to that of an ethyl group ( $V_{vdw} = 38.9 \text{ \AA}^3$ ) compared to an isopropyl group ( $V_{vdw} = 56.2 \text{ \AA}^3$ ; Zhao et al. 2003).

The head twitch response (HTR) is a rapid paroxysmal head movement induced by serotonergic hallucinogens in rats and mice due to 5-HT<sub>2A</sub> receptor activation (Canal and

Morgan 2012; Halberstadt and Geyer 2013). The HTR is commonly used as a behavioral proxy in rodents for human hallucinogenic effects because it can reliably distinguish between hallucinogenic and non-hallucinogenic 5-HT<sub>2A</sub> receptor agonists (Gonzalez-Maeso et al. 2007). LSD and analogs such as AL-LAD and LSZ induce HTRs in mice with high potency (Corne and Pickering 1967; Halberstadt and Geyer 2013; Brandt et al. 2017a). HTR studies were conducted in C57BL/6J mice to assess whether ECPLA activates 5-HT<sub>2A</sub> receptors and produces LSD-like behavioral effects *in vivo*. To compare the potency of ECPLA with other *N*-alkyl-substituted lysergamides in mice, additional experiments examined the effect of *N*-methyl-*N*-isopropyl lysergamide (MIPLA) and LAMPA in the HTR paradigm. These congeners have the same number of carbon atoms in the amide moiety and would have hydrophobicity virtually identical to LSD. In addition, in rats trained to discriminate saline from LSD, MIPLA was only slight less potent than LSD (0.036 mg/kg vs 0.021 mg/kg, respectively). Although MIPLA had 6-fold lower affinity than LSD at [<sup>3</sup>H]ketanserin-labeled sites in rat forebrain homogenate (28.1 vs. 4.78 nM, respectively), it had about 82% of the affinity of LSD at [<sup>125</sup>I]DOI-labeled sites (MIPLA  $K_i$  = 1.75 nM, LSD  $K_i$  = 1.43 nM; Huang et al. 1994). Importantly, MIPLA has been reported to have about one-third the potency of LSD as a psychedelic in man (Shulgin 2016). Recent online postings indicate that MIPLA is available as an NPS (e.g., Anonymous, 2018). By contrast, little is known about the pharmacology of LAMPA. In a study conducted in six hallucinogen-experienced subjects, administration of LAMPA (100 µg p.o.) had no effect in four subjects and produced effects consistent with a threshold dose of LSD in two subjects (Abramson and Rolo 1967).



## MATERIALS AND METHODS

### *Animals*

Male C57BL/6J mice (6-8 weeks old) obtained from Jackson Laboratories (Bar Harbor, ME, USA) were housed in a vivarium at the University of California San Diego, an AAALAC-approved animal facility that meets all Federal and State requirements for care and treatment of laboratory animals. Mice were housed up to four per cage in a climate-controlled room on a reverse-light cycle (lights on at 1900 h, off at 0700 h) and were provided with *ad libitum* access to food and water, except during behavioral testing. Testing was conducted between 1000 and 1800 h. All animal experiments were carried out in accordance with NIH guidelines and were approved by the UCSD animal care committee.

### *Drugs*

*N*-Ethyl-*N*-cyclopropyl lysergamide (ECPLA) was obtained from Synex Synthetics BV (Maastricht, The Netherlands) as the hemitartrate (2:1) salt. *N*-Methyl-*N*-propyl lysergamide (LAMPA) was obtained from Lipomed Inc. (Cambridge, MA, USA) as the freebase. *N*-Methyl-*N*-isopropyl lysergamide (MIPLA) freebase was available from previous studies. Competitive binding and functional assays were performed using ECPLA dissolved in dimethyl sulfoxide. For studies in mice, ECPLA was dissolved in isotonic saline and injected IP at a volume of 5 mL/kg. MIPLA and LAMPA were dissolved in 50 mM tartaric acid (pH ~5) and injected IP at 5 mL/kg.

### *Binding Studies*

A screening at 27 receptor binding sites was performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). ECPLA was tested at 10  $\mu$ M in competition assays against radioactive probe compounds; each primary binding assay was performed in quadruplicate. Sites exhibiting >50% inhibition at 10  $\mu$ M were tested in secondary assays at the identified receptor using 11 concentrations of ECPLA, measured in triplicate, to generate competition binding isotherms.  $K_i$  values were obtained from best-fit  $IC_{50}$  values (derived from nonlinear regression of the binding isotherms) using the Cheng-Prusoff equation (Cheng and Prusoff 1973). The radioligands used were as follows: [ $^3$ H]8-OH-DPAT (5-HT<sub>1A</sub>), [ $^3$ H]GR125743 (5-HT<sub>1B/1D</sub>), [ $^3$ H]5-HT (5-HT<sub>1E</sub>), [ $^3$ H]ketanserin (5-HT<sub>2A</sub>), [ $^3$ H]LSD (5-HT<sub>2B/5A/6/7</sub>), [ $^3$ H]mesulergine (5-HT<sub>2C</sub>), [ $^3$ H]prazosin ( $\alpha_{1A/1B/1D}$ ), [ $^3$ H]rauwolscine ( $\alpha_{2A/2B/2C}$ ), [ $^{125}$ I]pindolol ( $\beta_1$ ), [ $^3$ H]CGP12177 ( $\beta_2$ ,  $\beta_3$ ), [ $^3$ H]SCH23390 (D<sub>1</sub>, D<sub>5</sub>), [ $^3$ H]*N*-methylpiperone (D<sub>2/3/4</sub>), [ $^3$ H]pyrilamine (H<sub>1</sub>), [ $^3$ H]tiotidine (H<sub>2</sub>), [ $^3$ H] $\alpha$ -methylhistamine (H<sub>3</sub>), and [ $^3$ H]histamine (H<sub>4</sub>). The experimental protocols are available from the NIMH PDSP website (Roth 2013).

### *5-HT<sub>2</sub> Receptor Functional Assays*

Detailed protocols for the functional assays have been published online (Roth 2013). 5-HT<sub>2</sub> functional experiments (measuring Gq-mediated calcium flux) were performed in HEK293 cells stably expressing either human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, or 5-HT<sub>2C</sub> receptors. The day before the assay, cells were seeded into poly-L-lysine-coated black 384-well clear-bottom tissue culture

plates with DMEM (Invitrogen, Carlsbad, CA, USA) containing 1% dialyzed fetal bovine serum at a density of ~15,000 cells in 40  $\mu$ L per well. The next day, a stock solution of ECPLA was diluted in assay buffer (HBSS, 20 mM HEPES, 2.5 mM probenecid, pH 7.4). Media were decanted and replaced with 20  $\mu$ L per well of assay buffer containing Fluo-4 Direct dye (Invitrogen) and incubated for 1 h at 37°C. Plates were allowed to equilibrate at room temperature and  $\text{Ca}^{2+}$  flux was measured using a FLIPR<sup>TETRA</sup> system (Molecular Devices, Sunnyvale, CA, USA). Plates were read for fluorescence initially for 10 s (1 read per s) to establish a baseline, and then stimulated by the addition of 10  $\mu$ L/well of drug dilutions or buffer and read for an additional 120 s. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose-response function of GraphPad Prism 5.0.

### *Head Twitch Response Studies*

The head twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil (Halberstadt and Geyer 2013,2014; Nichols et al. 2015; Klein et al. 2018). Briefly, mice were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Test compounds were injected immediately prior to testing. Mice ( $n=5-7$ /group) were injected with drug or vehicle and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min.

Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, and digitized (20 kHz sampling rate) using a Powerlab/8SP with LabChart v 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified manually based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least three sequential head movements (usually exhibited as bipolar peaks) with frequency  $\geq 40$  Hz; 3) amplitude exceeding the level of background noise; 4) duration  $< 0.15$  s; and 5) stable coil voltage immediately preceding and following each response.

### *Data Analysis*

Head twitch counts were analyzed using one-way analyses of variance (ANOVA). *Post hoc* pairwise comparisons between selected groups were performed using Tukey's studentized range method. Significance was demonstrated by surpassing an  $\alpha$ -level of 0.05. The entire 30-min recordings were examined for head twitches, but in some cases a shorter block of time was used for analysis to accommodate compounds with a brief duration-of-action since potency calculations can be confounded by extended periods of inactivity. Median effective doses (ED<sub>50</sub> values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression (Prism 7.00, GraphPad Software, San Diego, CA, USA). A Gaussian distribution (Christopoulos et al. 2001) was used to fit biphasic HTR dose-response data:

$$E = \text{Baseline} + \text{Range} \times e^{-\left[\frac{|\log [A] - \text{midA}|}{\text{slope}}\right]^2}$$

$$\text{midA} = \log\text{ED}_{50} + \text{slope}\sqrt{-\ln(0.5)}$$

In these equations,  $E$  is the drug effect, *Baseline* is the response in the control group, *Range* is the distance from Baseline to the top of the curve,  $[A]$  is the dose of the drug, and *midA* is the logarithm of the dose corresponding to the top of the curve.

## RESULTS

Table 1 lists the affinities of ECPLA for 27 serotonergic, adrenergic, dopaminergic, and histaminergic receptors. The binding affinities and selectivity of ECPLA observed for monoaminergic sites are consistent with the values previously reported for LSD (Leysen 1989; Marona-Lewicka and Nichols 1995; Nichols et al. 2002; Rickli et al., 2015). ECPLA was observed to bind nonselectively to 5-HT receptors with affinities in the 1–100 nM range. The sites with the highest affinities were 5-HT<sub>1A</sub> ( $K_i = 3.2$  nM), 5-HT<sub>2B</sub> ( $K_i = 5.3$  nM), and 5-HT<sub>5A</sub> ( $K_i = 8.6$  nM). Using the same conditions, LSD was previously reported to bind to human 5-HT<sub>2A</sub> receptors (labeled with [<sup>3</sup>H]ketanserin) and human 5-HT<sub>2C</sub> receptors (labeled with [<sup>3</sup>H]mesulergine) with  $K_i$  values of 13 nM and 30 nM, respectively (Nichols 2017); compared to LSD, ECPLA has equivalent affinity for the 5-HT<sub>2A</sub> receptor ( $K_i = 16.5$  nM) but almost 3-fold lower affinity for the 5-HT<sub>2C</sub> receptor ( $K_i = 85.7$  nM). ECPLA binds to  $\alpha$ -adrenoceptors with some preference for  $\alpha_2$  subtypes compared to  $\alpha_{1A}$ ,  $\alpha_{1B}$ , and  $\alpha_{1D}$  sites. The affinity of ECPLA for  $\alpha_{2A}$  and  $\alpha_{2B}$  adrenergic sites ( $K_i$  values of 144 nM and 62.3 nM, respectively) approximates the reported affinity of LSD for  $\alpha_2$ -adrenoceptors labeled with [<sup>3</sup>H]clonidine ( $K_i = 37$ –82 nM) in rat

cortex (Leysen 1989; Marona-Lewicka and Nichols 1995). In contrast to LSD (Nichols et al. 2002), ECPLA did not bind to  $\beta_1$  or  $\beta_2$  sites with appreciable affinity (<50% displacement at 10  $\mu$ M), but did interact with  $\beta_3$  sites with affinity in the micromolar range ( $K_i = 2,415$  nM). Similar to LSD (Nichols et al. 2002; Rickli et al. 2015), ECPLA binds to dopamine receptors with some selectivity for  $D_2$ -like sites ( $D_2$ ,  $D_3$ , and  $D_4$  receptors) compared to  $D_1$ -like sites ( $D_1$  and  $D_5$  receptors). ECPLA has negligible affinity for  $H_1$ ,  $H_3$ , or  $H_4$  receptors in these experiments (i.e., <50% displacement at 10  $\mu$ M), whereas it binds to  $H_2$  receptors with a  $K_i$  of 890 nM. LSD reportedly binds to  $H_1$  receptors with a  $K_i$  of 1,100 to 1,500 nM (Nichols et al. 2002; Rickli et al. 2015) but its affinity for other histamine receptors has not been reported.

The effect of ECPLA on human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors was assessed using G<sub>q</sub>-mediated Ca<sup>2+</sup> mobilization in HEK293 cells (Table 2). As shown in Figure 2, ECPLA acted as a partial agonist at all three 5-HT<sub>2</sub> receptor subtypes. ECPLA has the highest potency and efficacy at 5-HT<sub>2A</sub> receptors ( $EC_{50} = 14.6$  nM;  $E_{max} = 91\%$ ) compared to 5-HT<sub>2B</sub> ( $EC_{50} = 36.7$  nM;  $E_{max} = 73\%$ ) and 5-HT<sub>2C</sub> ( $EC_{50} = 183$  nM;  $E_{max} = 73\%$ ) receptors.

ECPLA produced a significant increase in HTR counts over baseline levels in mice ( $F(3,20) = 14.92$ ,  $p < 0.0001$ ). Similar to other lysergamides (Halberstadt and Geyer 2013; Brandt et al. 2017a), ECPLA induced head twitches with a biphasic inverted-U-shaped dose-response function, with the peak response occurring at 0.3 mg/kg ( $p < 0.01$ , Tukey's test; see Figure 3). The  $ED_{50}$  of ECPLA is 130.2  $\mu$ g/kg (317.2 nmol/kg) with a 95% CI of 87.5–193.7  $\mu$ g/kg (213.2–471.9 nmol/kg). In comparison, LSD had an  $ED_{50}$  of 132.8 nmol/kg under equivalent conditions (Halberstadt and Geyer 2013), meaning that ECPLA induces the HTR with 42% of the molar potency of LSD.

As shown in Figure 3, MIPLA ( $F(3,17) = 36.12, p < 0.0001$ ) and LAMPA ( $F(4,23) = 14.44, p < 0.0001$ ) also induced the HTR in mice with biphasic dose-responses. The  $ED_{50}$  for MIPLA was 136.4  $\mu\text{g}/\text{kg}$  (421.7  $\text{nmol}/\text{kg}$ ) with a 95% CI of 116.6–159.5  $\mu\text{g}/\text{kg}$  (360.5–493.1  $\text{nmol}/\text{kg}$ ). The  $ED_{50}$  for LAMPA was 115.9  $\mu\text{g}/\text{kg}$  (358.3  $\text{nmol}/\text{kg}$ ) with a 95% CI of 78.0–172.2  $\mu\text{g}/\text{kg}$  (241.1–532.4  $\text{nmol}/\text{kg}$ ).

## DISCUSSION

The present investigation was conducted to examine the pharmacological properties of ECPLA, a novel analog of LSD. Studies assessed the binding of ECPLA to monoamine receptors and its ability to activate 5-HT<sub>2</sub> subtypes *in vitro*. Additional experiments were conducted to determine whether ECPLA induces the HTR in mice. ECPLA bound to 5-HT receptors, adrenergic  $\alpha_{2A}$  and  $\alpha_{2B}$  receptors, and D<sub>2/3/4</sub> dopamine receptors with moderately high affinity, which is reminiscent of the binding profile of LSD. ECPLA was nearly a full agonist for Gq-mediated calcium flux at the 5-HT<sub>2A</sub> receptor but had lower efficacy and potency at 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors. In addition, the effects of ECPLA in the head twitch paradigm suggest that it is capable of activating 5-HT<sub>2A</sub> receptors *in vivo*. In summary, the fact that ECPLA is a 5-HT<sub>2A</sub> agonist with nanomolar affinity and produces HTRs in mice strongly suggests it will act as an LSD-like (hallucinogenic) agent in humans.

ECPLA and LSD have remarkably similar binding profiles at monoamine receptors. Replacing one of the two ethyl groups present in LSD with a cyclopropyl group apparently has little influence on its ability to interact with serotonergic, dopaminergic, adrenergic, or histaminergic receptors. One notable difference between LSD and ECPLA is that the former

compound has moderate affinity for  $\beta_1$  ( $K_i = 140$  nM) and  $\beta_2$  ( $K_i = 740$  nM) receptors (Nichols et al. 2002) whereas the affinity of ECPLA for those receptors is negligible (i.e., <50% displacement at 10  $\mu$ M). Nevertheless, in humans, the concentration of LSD in cerebrospinal fluid (CSF) probably does not exceed 0.6–0.8 nM after a 200  $\mu$ g p.o. dose (Dolder et al. 2015), so the interaction between LSD and  $\beta$ -adrenergic receptors is unlikely to contribute to its psychopharmacology. Although the concentration of LSD in CSF is relatively low compared to its reported affinity for 5-HT<sub>2A</sub> ( $K_i = 13$  nM; Nichols 2017), the competitive binding assays used to determine the  $K_i$  are likely to underestimate the affinity of LSD due to its receptor kinetics. LSD has a long residence time at the 5-HT<sub>2A</sub> receptor; reported  $t_{1/2}$  values range from 20 min (Bennett and Snyder 1975; Burris and Sanders-Bush 1992) to 138.6 min (Wacker et al 2017). Given the slow dissociation of LSD from 5-HT<sub>2A</sub>, binding may not reach equilibrium during competitive binding assays, resulting in an underestimation of affinity. Indeed, when affinity is determined using association and dissociation assays, [<sup>3</sup>H]LSD binds to the 5-HT<sub>2A</sub> receptor with  $K_D = 0.33$  nM (Wacker et al. 2017), which is consistent with the concentration in CSF.

The binding of LSD to the 5-HT<sub>2A</sub> receptor is known to be highly sensitive to the steric and conformational properties of the amide substituent (Oberlender et al. 1992; Huang et al. 1994; Monte et al. 1995; Nichols et al. 1996). The conformation of the diethylamide moiety in LSD is optimal for binding because it interacts with a region of the 5-HT<sub>2A</sub> receptor that has a highly complementary structure (Wacker et al. 2017). ECPLA and LSD exhibited similar affinities for [<sup>3</sup>H]ketanserin-labeled 5-HT<sub>2A</sub> sites, indicating the binding site can readily accommodate an amide substituent that is marginally bulkier than ethyl. Although cyclopropyl substitution did not alter 5-HT<sub>2A</sub> binding affinity relative to LSD, a reduction of agonist potency at the 5-HT<sub>2A</sub> receptor was observed. ECPLA activates the human 5-HT<sub>2A</sub> receptor (h5-HT<sub>2A</sub>)



with an  $EC_{50}$  of 14.6 nM. According to Wacker et al. (2017), LSD activates  $h5-HT_{2A}$  in the same expression system with an  $EC_{50}$  of 3.61 nM. The difference in 5- $HT_{2A}$  agonist potency between LSD and ECPLA roughly parallels their potencies in the HTR assay, which is consistent with our previous finding that  $ED_{50}$  values in the HTR assay are correlated with potencies for inducing  $Ca^{2+}$  mobilization via the 5- $HT_{2A}$  receptor (Nichols et al. 2015). Therefore, compared to 5- $HT_{2A}$  binding affinity, receptor activation may exhibit a greater sensitivity to changes in the steric bulk of the amide substituent. In addition to increasing steric bulk, cyclopropyl substitution would also alter the conformational mobility of the amide side-chain. Homology modeling suggest that the two ethyl groups in LSD adopt a *trans* conformation when bound to the 5- $HT_{2A}$  receptor (Wacker et al. 2017); the substituents in ECPLA may adopt a slightly different orientation, potentially reducing agonist potency.

The reduction in *in vivo* potency produced by replacing one of the *N*-ethyl groups in LSD with an *N*-cyclopropyl group closely mirrored the effect of *N*-isopropyl substitution observed previously with EIPLA. According to Huang et al. (1994), EIPLA produced full substitution in rats trained to discriminate 0.08 mg/kg LSD but was only 36% as potent as the training drug. A similar potency reduction (42%) occurred with ECPLA relative to LSD in the HTR assay. Although many hallucinogens have been tested in both mouse head twitch and rat drug discrimination (DD) studies, it is not clear whether a relationship exists between potencies in these paradigms, making it difficult to compare the relative behavioral potencies of EIPLA and ECPLA. Nevertheless, according to our preliminary studies, there does appear to be a robust and highly significant correlation between rat DD- and mouse HTR-derived  $ED_{50}$  values for phenylalkylamine and tryptamine hallucinogens (Halberstadt et al., unpublished findings), demonstrating that potencies in the HTR and DD paradigms are likely closely related.

MIPLA, the *N*-methyl-*N*-isopropyl isomer of LSD, also induced head twitches in mice. On a molar basis, MIPLA was only slightly less potent than ECPLA in the HTR paradigm and has about a third of the potency of LSD. Since MIPLA and ECPLA have nearly comparable potencies in mice, it appears that *N*-cyclopropyl vs. *N*-isopropyl substitution does not appreciably alter the behavioral pharmacology of lysergamides. MIPLA was found to fully substitute for LSD in rats, with about half the potency of the training drug (Huang et al. 1994). According to Shulgin, human subjects administered MIPLA at doses of 180–300 µg experienced LSD-like psychedelic effects, making it about two- to threefold less potent than LSD (Shulgin 2016). Hence, there is converging evidence that MIPLA also has behavioral effects comparable to LSD but with reduced potency.

The LSD isomer LAMPA induced head twitches in mice and was virtually equipotent with ECPLA and MIPLA. Although LAMPA is reportedly less potent than LSD in humans (Abramson and Rolo 1967), few details have been published regarding the psychopharmacology of this compound. Based on the HTR data, LAMPA is predicted to act as a hallucinogen in humans with approximately three-fold lower potency than LSD.

The pharmacological and behavioral properties of ECPLA are thus reminiscent of LSD and other lysergamide hallucinogens. In this study, ECPLA acted as a potent and highly efficacious 5-HT<sub>2A</sub> agonist and induced head twitches in mice with only slightly lower potency than LSD. These findings indicate that ECPLA is likely to produce hallucinogenic effects in humans, although clinical trials are ultimately necessary to understand the human psychopharmacology of ECPLA. Given the pharmacology of ECPLA, it has the potential to be used in a manner similar to other lysergamide hallucinogens. LSD is becoming an important tool

in clinical research and the possibility exists that ECPLA could also be used for similar research purposes.

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Table 1. Receptor binding data for *N*-ethyl-*N*-cyclopropyllysergamide (ECPLA).

Receptor	Species <sup>a</sup>	Radioligand	$K_i$ (nM) <sup>b,c</sup>
5-HT <sub>1A</sub>	Human	[ <sup>3</sup> H]8-OH-DPAT	3.2 ± 0.5 (3)
5-HT <sub>1B</sub>	Human	[ <sup>3</sup> H]GR125743	42.3 ± 8.7 (3)
5-HT <sub>1D</sub>	Human	[ <sup>3</sup> H]GR125743	12.6 ± 1.9 (3)
5-HT <sub>2A</sub>	Human	[ <sup>3</sup> H]ketanserin	16.5 ± 6.0 (3)
5-HT <sub>2B</sub>	Human	[ <sup>3</sup> H]LSD	5.3 ± 1.3 (3)
5-HT <sub>2C</sub>	Human	[ <sup>3</sup> H]mesulergine	85.7 ± 31.2 (3)
5-HT <sub>5A</sub>	Human	[ <sup>3</sup> H]LSD	8.6 ± 4.2 (3)
5-HT <sub>6</sub>	Human	[ <sup>3</sup> H]LSD	21.7 ± 4.4 (3)
5-HT <sub>7</sub>	Human	[ <sup>3</sup> H]LSD	27.3 ± 11.5 (4)
α <sub>1A</sub>	Human	[ <sup>3</sup> H]prazosin	> 10,000
α <sub>1B</sub>	Human	[ <sup>3</sup> H]prazosin	1954 ± 152 (3)
α <sub>1D</sub>	Human	[ <sup>3</sup> H]prazosin	> 10,000
α <sub>2A</sub>	Human	[ <sup>3</sup> H]rauwolscine	144 ± 64 (3)
α <sub>2B</sub>	Human	[ <sup>3</sup> H]rauwolscine	62.3 ± 15.1 (3)
α <sub>2C</sub>	Human	[ <sup>3</sup> H]rauwolscine	438 ± 118 (3)
β <sub>1</sub>	Human (heart <sup>d</sup> )	[ <sup>125</sup> I]pindolol	> 10,000 <sup>e</sup>
β <sub>2</sub>	Human	[ <sup>3</sup> H]CGP12177	> 10,000 <sup>e</sup>
β <sub>3</sub>	Human	[ <sup>3</sup> H]CGP12177	2415 ± 639 (4)
D <sub>1</sub>	Human	[ <sup>3</sup> H]SCH23390	502 ± 122 (3)
D <sub>2</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	150 ± 32 (3)
D <sub>3</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	104 ± 29 (3)
D <sub>4</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	92.3 ± 11.9 (3)
D <sub>5</sub>	Human	[ <sup>3</sup> H]SCH23390	1504 ± 386 (4)
H <sub>1</sub>	Human	[ <sup>3</sup> H]pyrilamine	> 10,000 <sup>e</sup>
H <sub>2</sub>	Human	[ <sup>3</sup> H]tiotidine	890 ± 137 (3)
H <sub>3</sub>	Guinea pig	[ <sup>3</sup> H]α-methylhistamine	> 10,000 <sup>e</sup>
H <sub>4</sub>	Human	[ <sup>3</sup> H]histamine	> 10,000 <sup>e</sup>

<sup>a</sup>The experiments were performed using cloned receptors from the species indicated.

<sup>b</sup>Mean ± SEM.

<sup>c</sup>The number of independent determinations (performed in triplicate) are listed in parentheses.

<sup>d</sup>The experiment was performed using tissues natively expressing the receptor.

<sup>e</sup>IC<sub>50</sub> > 10 μM in the primary binding assay.

Table 2. Functional activity of *N*-ethyl-*N*-cyclopropyllysergamide (ECPLA) at 5-HT<sub>2</sub> receptors.

Receptor	ECPLA		5-HT	
	EC <sub>50</sub> , nM (pEC <sub>50</sub> ± SEM)	<i>E</i> <sub>max</sub> % 5-HT	EC <sub>50</sub> , nM (pEC <sub>50</sub> ± SEM)	<i>E</i> <sub>max</sub> % 5-HT
h5-HT <sub>2A</sub>	14.6 (7.84 ± 0.04)	91 ± 1	0.50 (9.30 ± 0.04)	100
h5-HT <sub>2B</sub>	36.7 (7.44 ± 0.06)	73 ± 2	0.56 (9.25 ± 0.03)	100
h5-HT <sub>2C</sub>	183 (6.74 ± 0.09)	73 ± 3	0.24 (9.63 ± 0.07)	100

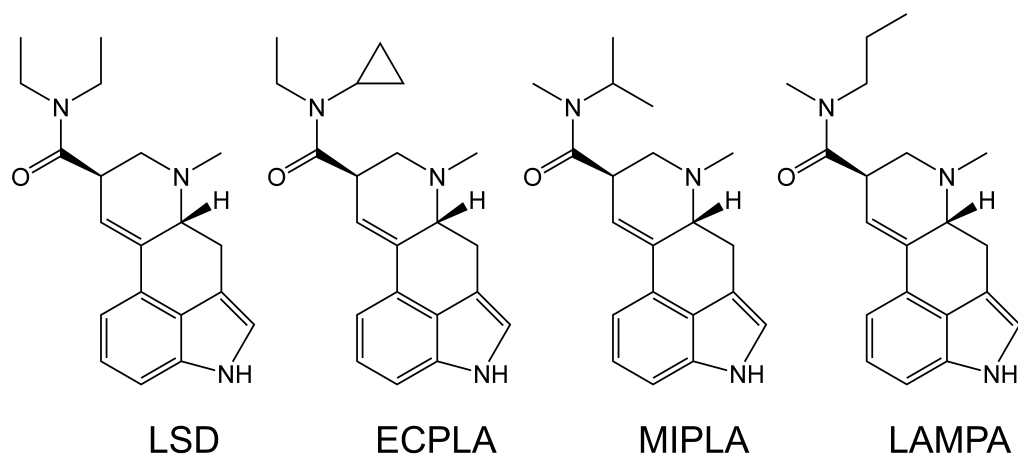
Data were acquired with HEK293 cells expressing either human 5-HT<sub>2A</sub> (h5-HT<sub>2A</sub>), human 5-HT<sub>2B</sub> (h5-HT<sub>2B</sub>), or human 5-HT<sub>2C</sub> INI (h5-HT<sub>2C</sub>) receptors. Data represent EC<sub>50</sub> and *E*<sub>max</sub> means and standard error of the mean (SEM) from two independent experiments performed in quadruplicate. *E*<sub>max</sub> is defined as percent 5-HT maximum response.

Table 3. Effect of lysergamides on the head twitch response (HTR).

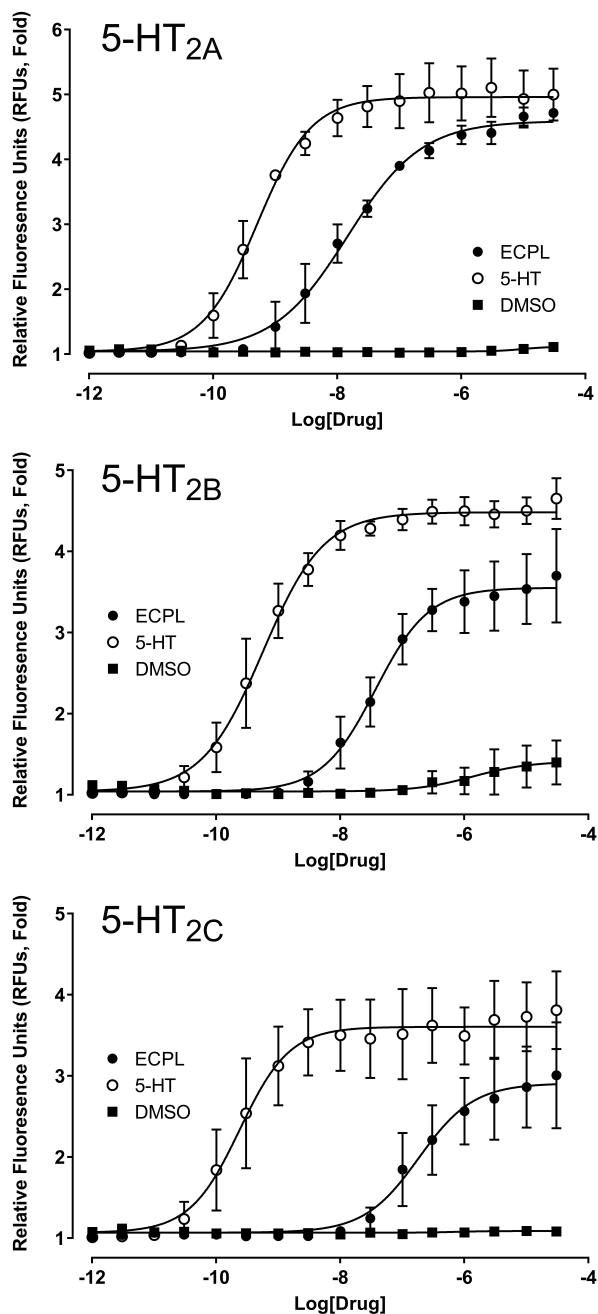
Compound	Dose (mg/kg)	<i>N</i>	Duration (min)	HTR Counts (Mean ± SEM)	ED <sub>50</sub> , µg/kg (95% CI)	ED <sub>50</sub> , nmol/kg (95% CI)
ECPLA <sup>a</sup>	0	6	30	7.3 ± 1.7	130.2	317.2
	0.1	7		17.7 ± 2.6	(87.5–193.7)	(213.2–471.9)
	0.3	5		34.6 ± 5.1 *		
	1	6		28.2 ± 2.6 *		
MIPLA <sup>b</sup>	0	5	30	6.2 ± 1.4	136.4	421.7
	0.1	6		12.3 ± 1.1	(116.6–159.5)	(360.5–493.1)
	0.2	5		31.8 ± 1.9 *		
	0.4	5		26.6 ± 3.1 *		
LAMPA <sup>b</sup>	0	6	20	5.0 ± 1.0	115.9	358.3
	0.1	5		15.8 ± 2.7	(78.0–172.2)	(241.1–532.4)
	0.3	6		31.0 ± 2.2 *		
	1	6		19.5 ± 3.2 *		
	3	5		7.4 ± 4.3		

<sup>a</sup>hemitartrate (2:1) salt; <sup>b</sup>free base. \**p*<0.01 vs. vehicle control group (Tukey's test).

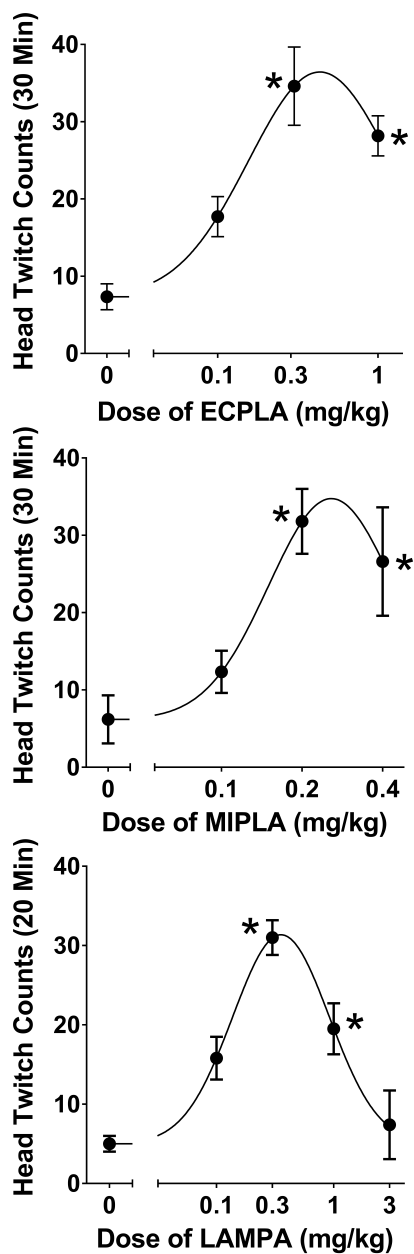
**FIGURE CAPTIONS**



**Figure 1.** Chemical structures of lysergic acid diethylamide (LSD), *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA), *N*-methyl-*N*-isopropyl lysergamide (MIPLA), and *N*-methyl-*N*-propyl lysergamide (LAMPA).



**Figure 2.** Agonist potencies of *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA) at 5-HT<sub>2</sub> receptor subtypes. Concentration-response curves for ECPLA and dimethylsulfoxide (DMSO) vehicle are shown for human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors expressed in HEK293 cells. Values are the mean  $\pm$  SEM of two independent experiments performed in quadruplicate.



**Figure 3.** Effect of *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA, top panel), *N*-methyl-*N*-isopropyl lysergamide (MIPLA, middle panel), and *N*-methyl-*N*-propyl lysergamide (LAMPA, bottom panel) on the head twitch response. Data are presented as group means  $\pm$  SEM for the first 20-min of the test session (LAMPA) or the entire 30-min test session (ECPLA and MIPLA). \* $p$ <0.01, significant difference from the vehicle control group (Tukey's test).