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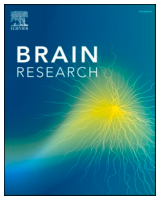

Research paper

Dimethyltryptamine (DMT) and ibogaine elicit membrane effects in HEK cells transiently transfected with the human 5-HT2A receptor

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ABSTRACT

Psychedelics show promise in treating psychiatric disorders. Therapeutic effects appear to involve activation of the 5-Hydroxytryptamine 2A receptor (5-HT_{2A}R), a G protein-coupled receptor (GPCR). Several SNPs of the 5-HT_{2A}R naturally occur, which are associated with differences in receptor function and altered responsiveness to treatments. New compounds suspected to act at the 5-HT_{2A}R are actively being generated. HEK cells are not commonly used to study membrane effects induced by agonists of GPCRs. In this study, for the first time, membrane actions of two psychedelics, dimethyltryptamine (DMT) and ibogaine on HEK cells transiently transfected with either the human wildtype (WT) or the human I197V mutated 5-HT_{2A}R were investigated using whole-cell electrophysiology. Membrane effects were observed in both genotypes and with both drugs in most cells, while no responses were observed in non-transfected HEK cells suggesting that responses were due to 5-HT_{2A}R activation. In HEK cells transfected with the I197V SNP, a significantly shorter duration of the DMT response was observed, however there were no differences in drug-elicited amplitudes between drug or receptor genotype. I-V curves showed a significant effect of drug exposure for both DMT and ibogaine at the highest concentration evaluated. Taken together, our data show transfection of the 5-HT_{2A}R, a GPCR, in HEK cells is able to activate downstream ion channels following exposure to two different 5-HT_{2A}R agonists. Accordingly, investigations of novel compounds suspected to act at 5-HT_{2A}Rs can include examination of elicitation of ionic currents in 5-HT_{2A}R transfected HEK cells, and drug effects at SNPs can also be evaluated.

1. Introduction

Cell lines are essential tools in biomedical research, providing a simplified, controlled environment to study cellular functions, drug responses, and disease mechanisms. In particular, they are valuable for high-throughput screening of potential therapeutic compounds, allowing scientists to rapidly assess e.g. drug efficacy and/or toxicity in a reproducible manner (Xu et al., 2020; Mohiuddin et al., 2021; Wei et al., 2021). Among the plethora of cell lines available, human embryonic kidney cells (HEK) cells are one of the most commonly used because of their rapid proliferation, high transfection efficiency, and stable growth characteristics. Further, HEK cells are capable of expressing recombinant proteins, which includes a wide array of receptors, and thus, this cell line is particularly favored in studies involving receptor pharmacology, including effects on receptor function of sequence variations in receptor genes. HEK cells also endogenously express a number of ion channels, namely functional voltage-gated K^+ (Avila et al., 2004; Yu and Kerchner, 1998; Jiang et al., 2002), Na^+ (Cummins et al., 1993), Cl^- (Zhu et al., 1998), Ca^{2+} (Berjukow et al., 1996) and non-selective cation (NSCC) channels (Zhu et al., 1998; Ye et al., 1996), which can enable the membrane of HEK cells to exert both inward and outward currents in response to their activation or inhibition, either directly or subsequent

Abbreviations: 5-HT2A receptor, Serotonin 2A receptor; ABSS, Artificial balanced salt solution; cDNA, Complementary deoxyribonucleic acid; DAG, Diacylglycerol; DMEM, Dulbecco's modified Eagle's medium; DMT, Dimethyltryptamine; DOM, 4-methoxy-2,5-dimethoxyphenylisopropylamine; EGTA, Ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; GFP, Green fluorescent protein; GPCR, G Protein-coupled receptor; hCB_1, Human cannabinoid receptor1; HEK cells, Human embryonic kidney cells; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IP_3, Inositol triphosphate; iPSC, induced pluripotent stem cell; I-V curve, Current-Voltage curve; NSCC, Non-selective cation channels; PIP_2, Phosphatidylinositol; PKC, Protein kinase C; PLC-β, Phospholipase C beta; SERT, Serotonin transporter; SNP, Single nucleotide polymorphism; TAAR1, Trace amine associated receptor; WT, Wildtype.

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to receptor activation (Berjukow et al., 1996; Zhu et al., 1998; Varghese et al., 2006).

Recently, psychedelics including compounds like N, N-dimethyltryptamine (DMT) and ibogaine have gained attention for their potential therapeutic effects in the treatment of psychiatric disorders such as depression. Among other targets, many of the classic psychedelics being evaluated for therapeutic actions activate the 5-Hydroxytryptamine 2A receptor (5-HT_{2A}R), which is also believed to be involved in the hallucinatory experience associated with use of psychedelics (Vollenweider et al., 1998; López-Giménez and González-Maeso, 2018; Halberstadt and Geyer, 2011; González-Maeso et al., 2007). While the necessity of activation of the 5-HT_{2A}R (Moliner et al., 2023), and the requirement of the hallucinogenic experience for psychiatric benefit is debated and unclear at this time (von Rotz et al., 2023; Sloshower et al., 2023; Sekssaoui et al., 2024; Rosenblat et al., 2023; Gukasyan et al., 2022), much of the focus on development of second-generation anti-depressant compounds inspired by the actions of the psychedelics has been placed on synthesizing 5-HT_{2A}R agonists. The 5-HT_{2A}R is a G protein-coupled receptor (GPCR), which has been shown to modulate several downstream signaling pathways, including those involving β-arrestin and G_q (Guiard and Giovanni, 2015). β-arrestin and G_q are multifunctional proteins that initiate distinct signaling cascades, e.g. G_q initiates phospholipase C beta (PLC-β) mediated recruitment of inositol trisphosphate (IP_3) and diacylglycerol (DAG) from the membrane lipid, phosphatidylinositol bisphosphate (PIP_2) (Hoyer et al., 1994). Increased IP_3 stimulates the release of intracellular calcium from the endoplasmic reticulum and DAG activates protein kinase C (PKC), which can phosphorylate a wide range of ion channels including several voltage-dependent ion channels and NSCCs (Gada and Logothetis, 2022; Tsagareli, 2020). Further, activity of ion channels including NSCCs is also regulated by Ca^{2+}, PIP_2, DAG and PLC (Tsagareli, 2020; Partridge and Swandulla, 1988).

Recognition of the potential of agonists of the 5-HT_{2A}R, in combination with AI technology guiding structure-based drug design has led to an explosion in development of novel compounds, which could have actions at the 5-HT_{2A}R. Many of these compounds have been virtually screened but await functional screening (Zięba et al., 2024). HEK cells transfected with the 5-HT_{2A}R have been useful to evaluate the cellular effects of novel 5-HT_{2A}R agonists (Jensen et al., 2017), and further, can be used to evaluate the effects of these agonists at single nucleotide polymorphism (SNPs) in the 5-HT_{2A}R, which have been associated with an altered responsiveness to anti-depressive and anti-psychotic medication (Schmitz et al., 2022; Peters et al., 2004; Lucae et al., 2010; Lin et al., 2009; Davies et al., 2006). Currently, assays based on detection of changes in calcium, cAMP, β-arrestin and G_q have been used in HEK cells transfected with the 5-HT_{2A}R to evaluate activity stimulated by agonism of the receptor (Schmitz et al., 2022; Hagberg et al., 1998; Day et al., 2002; Gray et al., 2001), and HEK cells transfected with several cDNA plasmids coding for SNPs have been used to screen differences in receptor pharmacology of various psychedelics (Schmitz et al., 2022). However, to the best of our knowledge, there has not been a report where evaluation of membrane effects linked to activation of membrane channels has been conducted in HEK cells transfected with the 5-HT_{2A}R receptor, nor with sequence variants of the 5-HT_{2A}R gene. This could be because while membrane responses can readily be seen in response to agonists of transfected ionotropic receptors, transfected GPCRs may not be capable of activating ion channel effectors. However, in a previous study, agonism of transfected cannabinoid receptors was able to stimulate potassium and calcium currents in HEK cells (Vásquez et al., 2003). Further, stimulation of changes in calcium, cAMP, β-arrestin and G_q following agonism of the 5-HT_{2A}R in HEK cells indicate that intracellular machinery that could be linked to activation of ion channels induced by 5-HT_{2A}R stimulation is functional. Taken together, previous work suggested to us that transfection of HEK cells with 5-HT_{2A}R receptors could result in membrane effects involving activation of HEK cell native channels, and further, comparing these effects with those elicited by agonism of a commonly occurring SNP of the 5-HT_{2A}R could reveal differential effects.

Accordingly, this study aims to explore whether two classic psychedelics known to activate 5-HT_{2A}R (McKenna et al., 1990; Helsley et al., 1998) exert membrane effects on HEK cells transfected with the wildtype (WT) 5-HT_{2A}R. Further, we wished to determine whether differential actions were elicited in the I197V SNP of the 5-HT_{2A}R as responses of variants of this receptor class have been shown to be drug-specific. To the best of our knowledge, this is the first report evaluating the effect of 5-HT_{2A}R agonist activation on the membrane currents in transiently transfected HEK cells and on membrane currents elicited by agonist of a SNP 5-HT_{2A}R known to be associated with depression.

2. Methods

2.1. Culturing cells and transient transfection

HEK cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing GlutaMAX-I (ThermoFisher Scientific, Roskilde, Denmark) complemented with 10 % FBS (ThermoFisher Scientific, Roskilde, Denmark) and 1 % penicillin–streptomycin (ThermoFisher Scientific, Roskilde, Denmark) and incubated at 5 % CO_2 and 37 °C. Cells were split whenever 80–100 % confluency was reached and transfected accordingly. The transfection solution was prepared according to the manufacturer's protocol (TargetingSystems, 2019). The human cDNA coding for the 5-HT_{2A} wildtype (WT) (Genscript, Piscataway, NJ, USA) and I197V SNP mutation receptor (Genscript, Piscataway, NJ, USA) were transiently transfected into HEK cells. Importantly, it had previously been shown that transfection with I197V SNP in HEK cells does not result in meaningful pharmacological actions due to differences in receptor expression (Schmitz et al., 2022).

In brief, the transfection solution was prepared by adding 3 μg of DNA containing pcDNA3.1(+) plasmids of either 5-HT_{2A} WT or I197V SNP receptor (Genscript, Piscataway, NJ, USA), 3 μg of green fluorescent protein (GFP) plasmid (Provided by Anders A. Jensen) and 6 μL of Targefect-293 (Targeting systems, El Cajon, CA, USA) to 250 μL of DMEM, which were incubated for 20 min before adding the transfection solution to the 35 mm petri dish containing the HEK cells and incubated overnight. Cells with passage numbers between 9 and 42 were exclusively used in these experiments and electrophysiological recordings were conducted on the cells 24–48 h post transfection.

2.2. Whole-cell patch clamp electrophysiology on cultured cells

The culture medium containing HEK cells transiently transfected with either WT or I197V 5-HT_{2A}R, was replaced with Artificial balanced salt solution (ABSS) at room temperature (20–24 °C) and the 35 mm petri dish was placed on an Axiovert 10 microscope (Zeiss, Germany). The ABSS solution consisted of (in mM): 140 NaCl, 3.5 KCl, 1.25 Na_2HPO_4, 2 $MgSO_4$ (Merck, Søborg, Denmark), 2 $CaCl_2$ (Merck, Søborg, Denmark), 10 Glucose (VWR, Søborg, Denmark), and 20 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (TH. Geyer, Ballerup, Denmark) at pH: 7.35. Cells were observed through 200x magnification, and cells expressing GFP were visualized with a Xenon lamp (XBO 75 W/2; Zeiss, Germany). Micropipettes (1.5 mm OD Glass) (World Precision Instruments, Sarasota, FL, USA) used to patch HEK cell membranes were pulled to obtain a 2.0–4.0 MΩ resistance on a vertical microelectrode puller Model PP-830 (Narishige, Tokyo, Japan). Pipettes were filled with intracellular solution containing (in mM): 140 KCl, 1 $MgCl_2$ (VWR, Søborg, Denmark), 1 $CaCl_2$, 10 ethylene glycol-bis (β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) (Hello bio, Dunshaughlin, Ireland), 2 MgATP (Merck, Søborg, Denmark), and 10 HEPES at pH: 7.3. A seven-barrel application system (List, Darmstadt, Germany) was used to constantly apply ABSS or ABSS with either DMT or ibogaine in close proximity to the patched cell. Whole-cell, voltage clamp recordings were conducted using an EPC-9 amplifier (HEKA, Lambrecht, Germany). Cells were held at a potential of −70 mV and a 70 % compensation of the

series resistance was conducted during recordings of changes in membrane currents during application of DMT or ibogaine, and during voltage steps applied in order to obtain current–voltage relationship (I-V) curves. Changes in the current necessary to hold the cell at −70 mV were monitored and compared before and at the maximum deflection following 60 sec of drug application. Changes in this current exceeding 50 pA were considered a drug response. The amplitude of the current was measured from the start of the deflection to the plateau at the maximum deflection. Likewise, the duration of the response was measured as the time from the start of the deflection to return to baseline or when the response reached a new plateau. The duration included a natural lag-phase in the response initiation that is typical of activation of GPCRs. The I-V curves were obtained by stepping the cell from the holding potential to a series of incrementing voltages (from −110 mV to +20 mV with +10 mV increments) with each step lasting 300 ms before stepping back to the holding potential for at least 50–200 ms before launching the next step. I-V curves were obtained before and after exposure to DMT or ibogaine. Collection and analysis of the electrophysiological data were conducted using Pulse and PulseFit software 8.8 (HEKA, Lambrecht, Germany), respectively, and figures were made using Igor Pro software 6.20 (Wavemetrics, Portland, OR, USA) and Graphpad Prism™ 9.0 (San Diego, CA, USA).

2.3. Drugs

Dimethyltryptamine (DMT: Cayman chemical, Ann Arbor, MI, USA) was applied at 500 nM. Ibogaine (Chiron, Trondheim, Norway) was applied at two different concentrations, 30 or 100 μM to reflect potential differences exerted by this drug within a narrow range (Zetler et al., 1972; Repke et al., 1994; Callaway et al., 1999; Helsley et al., 1998). Both DMT and ibogaine powder were dissolved in 70 % ethanol and DMSO, respectively, for complete dissolution and their stock concentration were made so that when adding ABSS up to a total of 10 mL, the stocks were diluted 1000x at their final concentrations.

2.4. Statistical analysis

Possible differences in the response proportions between the WT and I197V were evaluated for the two drugs using a two-tailed Fischer's exact test. Following normality testing using the Shapiro-Wilk test, comparison of the amplitudes and durations of the inward currents were compared using either an unpaired *t*-test for datasets following normal distribution, or the Mann-Whitney test (MW-test) if data were not normally distributed. Voltage dependence of the current was evaluated using a three-way repeated measures ANOVA (voltage × drug × mutation), using data collected when each cell (either transfected with WT or I197V mutant) was subjected to the I-V protocol both in the absence and presence of drug. All statistical analyses were done using GraphPad Prism ™ 9.0 (San Diego, CA, USA). The number of responses in the population of cells tested is presented as fractions (Responding cells/total cells tested) and the polarity of the current responses are presented as a ratio of inward/outward currents elicited in responding cells.

3. Results

3.1. Membrane responses were observed for both ibogaine and DMT in WT and I197V mutated 5-HT_{2A}R

While DMT and ibogaine can activate 5-HT_{2A}R receptors, they are known to activate other transporter/receptors (e.g. Serotonin transporter (SERT), adrenergic and sigma receptors (Sweetnam et al., 1995; Greene, 2022; Alper, 2001), therefore, our first step was a control experiment to evaluate whether DMT and ibogaine could elicit a membrane response in non 5-HT_{2A}R transfected HEK cells. In a population of GFP-absent cells (Fig. 1 A1-2, blue arrow), 500 nM DMT (n=12) and 100 μM ibogaine (n=13) failed to elicit any response of the membrane (Fig. 1 B1-2).

After establishing that in naïve HEK cells, no membrane responses to DMT or ibogaine were elicited, we applied DMT and ibogaine to cells transfected with the WT 5-HT_{2A}R as indicated by presence of GFP-fluorescence (Fig. 1 A1-2, white arrow). In cells transfected with WT 5-HT_{2A}R, we elicited membrane responses in the majority of cells (DMT: n= 27/36 (75.0 %); 30 μM ibogaine: n= 23/25 (92.0 %); 100 μM ibogaine: n= 23/32 (71.9 %)). To provide evidence that effects were likely due to the transfection with the 5-HT_{2A}R, we applied a nonspecific 5-HTR agonist, 5-HT, and found that 30 μM of 5-HT elicited membrane responses in the majority of cells (n= 4/6; Fig. 1 B3). We then evaluated whether we could elicit membrane responses in cells transfected with the I197V SNP and found that membrane responses were elicited in the majority of GFP-positive cells (DMT: n= 15/22 (68.2 %); 30 μM Ibogaine: n= 9/11 (81.8 %); 100 μM ibogaine: n= 13/24 (54.2 %)). There were no differences when comparing the proportion of cells responding to DMT or ibogaine at either concentration between the two genotypes (Two-tailed Fischer's exact test; DMT: p= 0.7628, ibogaine 30 μM: p= 0.5705 and ibogaine 100 μM: p= 0.2598; Fig. 1 C1-3).

In the population of cells transfected with the WT or the I197V 5-HT_{2A}R receptor that responded to DMT, the majority of responses were inward currents, whereas in a minority of cells, outward currents were seen (Fig. 1 D1) (WT: n= 20/7 (74.1 %); I197V: n= 14/1 (93.3 %)). Ibogaine at both concentrations also elicited both inward or outward currents at both the WT and the I197V 5-HT_{2A}R, with the majority being inward currents (Fig. 1 D2-3) (Ibogaine 30 μM: WT: n= 22/1 (95.7 %); I197V: n= 7/2 (77.8 %); Ibogaine 100 μM: WT: n= 18/5 (78.3 %); I197V: n= 9/4 (69.2 %)). There were no differences in the distribution of the polarity of the response to DMT or ibogaine between the two genotypes (Two-tailed Fischer's exact test; DMT: p= 0.2225, ibogaine 30 μM: p= 0.1839 and ibogaine 100 μM: p= 0.6933; Fig. 1 D1-3).

As the majority of responses to both DMT and ibogaine in both genotypes were inward currents (Fig. 2 A1-3), we compared the amplitude and duration of this response type between HEK cells transiently transfected with either the WT or the I197V SNP receptor variant to see if differences in the response kinetics of inward currents were present between the two genotypes.

The average amplitude of the inward current induced by DMT in HEK cells transfected with WT was 787.1 ± 283.1 pA, and the average amplitude was 303.9 ± 55.4 pA in cells transfected with the I197V SNP receptor. This difference was not significant (Mann-Whitney (MW) test; p= 0.2642; Fig. 2 B1). The average amplitude of the inward current induced by ibogaine at 30 μM and 100 μM in the WT was 299.8 ± 71.8 pA and 265.5 ± 75.0 pA, respectively and in the I197V SNP, 30 and 100 μM ibogaine induced an average amplitude of inward current of 236.3 ± 83.9 pA and 396.4 ± 104.7 pA, respectively. The amplitude means were not significantly different between genotypes (Ibogaine 30 μM: MW test; p= 0.8577; Ibogaine 100 μM: MW test; p= 0.2854; Fig. 2 B2-3). The duration of the response did not differ between WT and the I197V SNP for either concentration of ibogaine (WT 30 μM: 135.0 ± 20.2 sec; I197V SNP 30 μM: 184.2 ± 30.3 sec, MW test; p= 0.2084; WT 100 μM: 169.1 ± 38.0 sec; I197V SNP 100 μM: 118.0 ± 25.4 sec, MW test; p= 0.5207; Fig. 2 C2-3)). However, there was a significant difference in duration in response to DMT between receptor genotypes as the duration was much shorter in cells transfected with the I197V SNP (Unpaired *t*-test; WT: 226.7 ± 28.9 sec; I197V: 107.7 ± 19.2 sec, p= 0.0022; Fig. 2 C1).

3.2. I-V curves

Induction of an inward current suggested that DMT and ibogaine were activating a conductance in HEK cells transfected with either the WT 5-HT_{2A} receptor, or the I197V SNP, and thus we wished to determine if we could characterize this conductance. Accordingly, we constructed I-V curves in both genotypes by stepping the membrane to various voltages from the holding voltage of −70 mV both in the presence and absence of drugs. For both DMT and ibogaine, we noted a drug induced

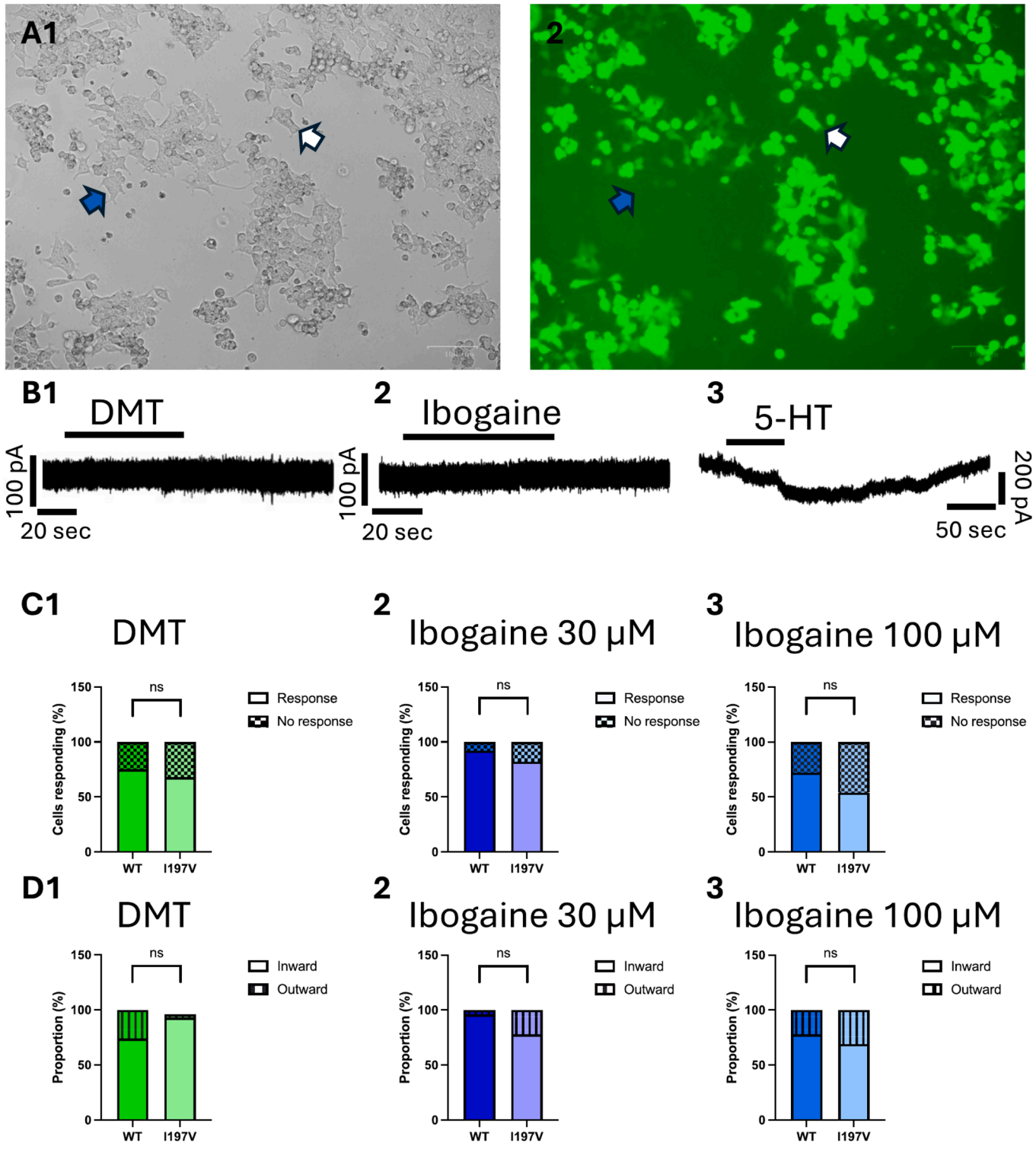

Fig. 1. DMT and ibogaine, as well as serotonin induced inward currents in 5-HT_{2A}R transfected cells. A1) Human embryonic kidney (HEK) cells visualized under bright-field illumination and A2) under ultraviolet (UV)-light, which excites the green fluorescent protein (GFP) present in transfected cells (white arrows). Non-transfected cells which lack expression of GFP are indicated with blue arrows in both images. Scale bar indicates 100 μm. B1) As shown in these representative responses in 3 different HEK cells, application of 1) dimethyltryptamine (DMT) or 2) ibogaine (100 μM) to GFP-absent, non-transfected HEK cells failed to elicit a membrane response, whereas 3) application of 30 μM of serotonin (5-HT) elicited inward currents in GFP-positive HEK cells transfected with the 5-HT_{2A}R Wildtype (WT) (GFP+). C) Population data are shown of the proportion of GFP-positive cells transfected with either the 5-HT_{2A}R WT or the I197V mutation, which responded to 1) DMT or two different concentrations of ibogaine: 2) 30 μM and 3) 100 μM. There were no statistical differences in the proportion of cells responding when compared across genotypes (Two-tailed Fischer's exact test; DMT: $p = 0.7628$, ibogaine 30 μM: $p = 0.5705$ and ibogaine 100 μM: $p = 0.2598$). D) The proportion of inward to outward currents in the population of responding cells are shown for 1) DMT, 2) ibogaine 30 μM and 3) 100 μM, where it can be seen that the distribution of response polarity did not statistically differ (Two-tailed Fischer's exact test; DMT: $p = 0.2225$, ibogaine 30 μM: $p = 0.1839$ and ibogaine 100 μM: $p = 0.6933$).

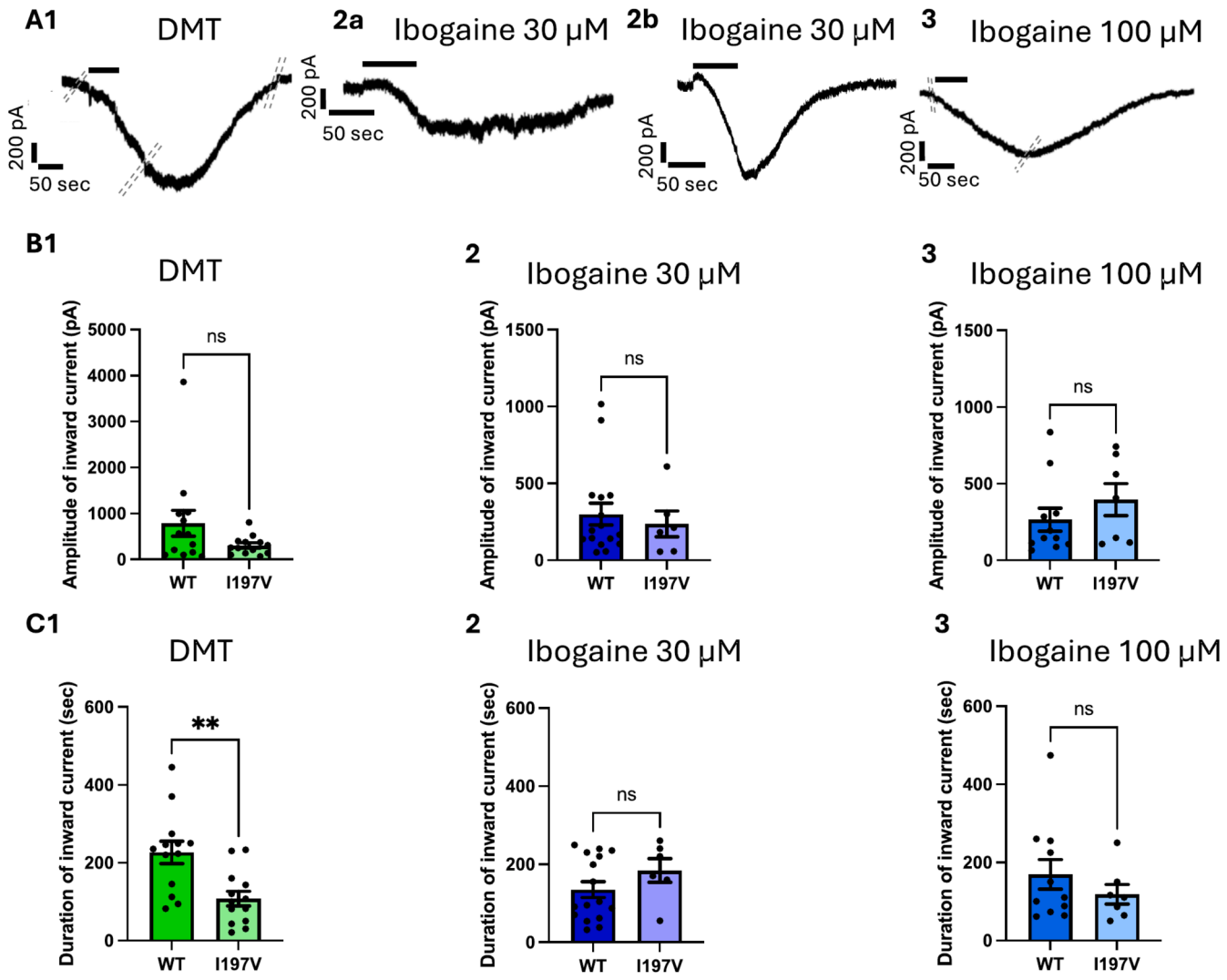

Fig. 2. Dimethyltryptamine (DMT) and ibogaine elicited inward membrane currents in wildtype and I197V 5-HT$_{2A}$R transfected HEK cells. A) Representative membrane responses of transfected human embryonic kidney (HEK) cells showing elicitation of inward currents by 1) DMT, 2a, b) 30 μM and, 3)100 μM ibogaine. B) When evaluating amplitudes across the population of HEK cells, the amplitude of the inward currents observed when applying 1) DMT (Mann-Whitney test (MW-test); DMT: p = 0.2642 and 2) Ibogaine 30 μM (MW-test; ibogaine 30 μM: p = 0.8577) and 3) 100 μM (MW-test; ibogaine 100 μM: p = 0.2854) did not differ between 5-HT$_{2A}$R Wildtype (WT) or the I197V mutation. C1) However, comparison of the duration of the response from the population of cells transfected with the 5-HT$_{2A}$R WT or the I197V mutation revealed a significant difference when DMT was applied (Unpaired *t*-test; DMT: p = 0.0022), whereas there were no significant genotype differences with 2) 30 μM ibogaine (MW-test; Ibogaine 30 μM: p = 0.2084) or 3) 100 μM (MW-test: ibogaine 100 μM: p = 0.5207). Dashed lines in A represent where I-V curves were obtained during the recording.

shift in the holding currents necessary to achieve the voltage steps in both WT and I197V transfected cells, which provided further support that the two drugs were exerting a membrane effect in both genotypes (Fig. 3A). As expected, a significant main effect of the voltage steps was revealed by the three-way ANOVA in both genotypes for DMT and ibogaine at both concentrations (Three-way ANOVA; DMT, 30 μM and 100 μM ibogaine: p<0.0001; Figs. 3 A1-3, B1-3). In addition, significant interactions between voltage and drugs were detected (Three-way ANOVA; DMT: p= 0.0003, 30 μM ibogaine: p= 0.0399, 100 μM ibogaine: p= 0.0056) reflecting that the effect of the drug on conductance increased at the more negative membrane potentials. A significant main (i.e. independent of membrane potential) effect of drug application was seen for DMT (Three-way ANOVA; p= 0.0227) and 100 μM ibogaine (Three-way ANOVA; p= 0.0300) but not 30 μM ibogaine (Three-way ANOVA; p=0.0538). No other significant main effects or interactions were detected, which indicates that there was no significant effect of the I197V mutation (Supplemental Table A).

Interception with the voltage axis (at I=0) of the control curves along with the curves obtained following application of DMT or ibogaine revealed a reversal potential of the drug-induced current. The DMT-induced current did not show a significant difference in reversal potential between genotypes (WT: −3.9 ± 2.9 mV; I197V: −11.1 ± 10.2 mV; MW test: p=0.9885). Similarly, the reversal potential for the current induced by 30 μM ibogaine did not differ according to genotype (WT: −30.2 ± 10.6 mV; I197V: 4.8 ± 5.1 mV, MW test: p= 0.1390). At the higher concentration of ibogaine, the average reversal potential also was not significantly different between genotypes (WT: −17.8 ± 6.4 mV; I197V: −7.6 ± 4.0 mV, MW test; p= 0.4086).

4. Discussion

DMT and ibogaine at two different concentrations elicited membrane responses in the majority of the HEK cells transfected with the WT or I197V SNP version of the 5-HT2A receptor. Further, when applied to WT transfected HEK cells, 5-HT also elicited membrane currents in the majority of the tested cells. Membrane responses were not elicited in

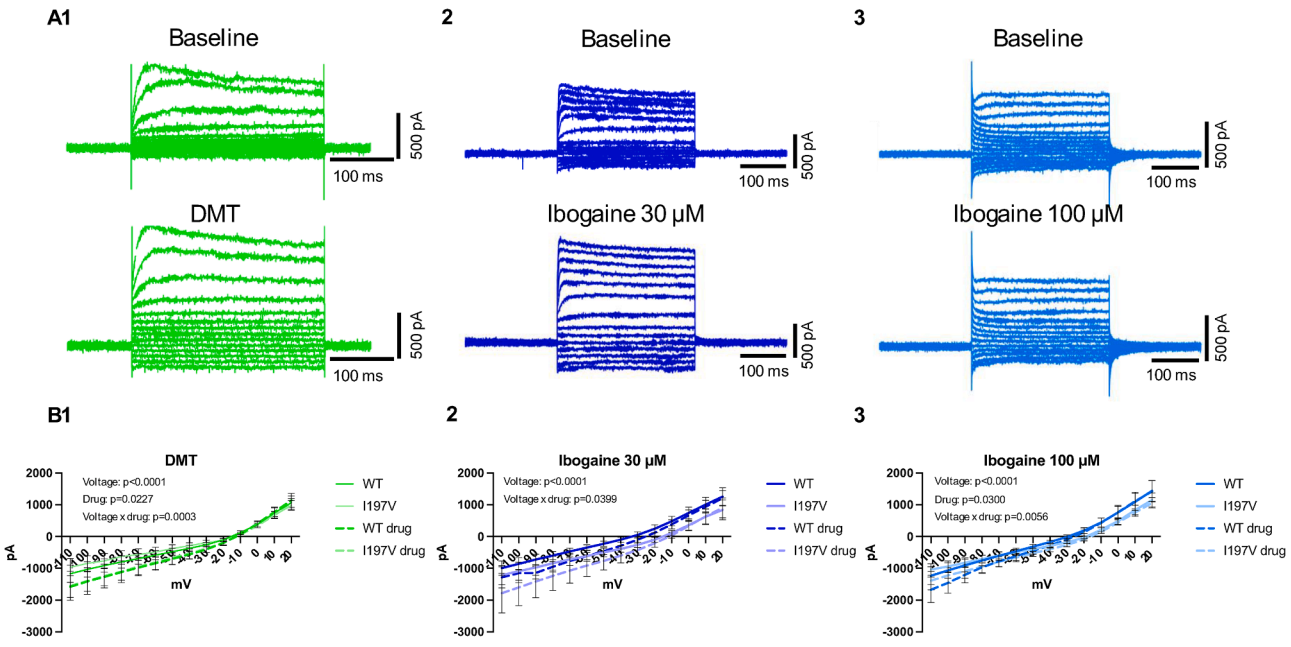

Fig. 3. DMT and ibogaine induce differential I-V relationships in 5-HT$_{2A}$ −expressing cells. A) Representative I-V curves generated by stepping the membrane potential from baseline to potentials ranging from −110 to + 20 mV in + 10 pA increments before, and after 60 sec of applying 1) DMT, 2) 30 μM or 3) 100 μM of ibogaine from three different WT 5-HT$_{2A}$ transfected HEK cells patched in whole-cell voltage-clamp configuration. B) I-V curves generated from the population of cells showed that the mean currents in the population of wildtype (WT) (n = 20) and I197V (n = 13) transfected HEK cells before and after DMT showed significant voltage and drug main effects (Three-way ANOVA; Voltage: $p < 0.0001$ and Drug: $p = 0.0227$) and also showed an interaction between voltage and drug (Three-way ANOVA; Voltage × Drug: $p = 0.0003$). A main effect in voltage was seen when applying 30 μM ibogaine in WT (n = 12) and I197V (n = 5) (Three-way ANOVA; $p < 0.0001$) and there was a significant interaction between voltage and drug (Three-way ANOVA; $p = 0.0399$). Main effects were noted of both voltage and drug when 100 μM ibogaine was applied in the WT (n = 13) and in I197V (n = 13) (Three-way ANOVA; Voltage: $p < 0.0001$ and Drug: $p = 0.0300$), again with a significant interaction between voltage and drug (Three-way ANOVA; Voltage × Drug: $p = 0.0056$).

non-transfected HEK cells. Taken together, our data strongly suggest that membrane responses observed following DMT or ibogaine were due to the activation of functional 5-HT$_{2A}$ receptors. This is, to the best of our knowledge, the first study to show membrane effects of DMT and ibogaine on HEK cells transfected with 5-HT$_{2A}$R.

While both inward and outward currents were elicited in HEK cells transfected with either the WT or I197V version of the 5-HT$_{2A}$ receptor, the majority of the responses were inward currents in both genotypes, and so we choose to evaluate the kinetics of this response type. The I197V mutation did not influence the amplitude of the responses when applying either DMT or ibogaine at the two concentrations. The duration of the inward current induced by DMT was significantly shorter in the HEK cells transfected with the I197V mutation compared to those transfected with WT. Evaluation of voltage steps in the HEK cells revealed that the inward current elicited by DMT was accompanied by an increase in conductance. Further, analysis of I-V plots revealed a greater enhancement in DMT-induced conductance at the more negative holding potentials. The reversal potential of the DMT induced conductance was approximately −3 and −11 mV in HEK cells transfected with the WT and I197V mutation, respectively. Interestingly, ibogaine exerted an effect on conductance at the 100 μM concentration, but at the lower concentration of 30 μM, we did not note a main effect of the drug on conductance but a significant interaction with voltage was seen. The ibogaine conductance had a reversal potential of approximately −30 mV and 4 mV in HEK cells transfected with the WT and I197V mutation, respectively. When taken together, our data are consistent with the interpretation that both DMT and ibogaine activate a NSCC in HEK cells transfected with the 5-HT$_{2A}$R. The 5-HT$_{2A}$ receptor is linked to G$_q$ which in turn produces several regulators that are linked to activation of NSCCs in other cell types (Tsagareli, 2020; Partridge and Swandulla, 1988). The NSCC is present in HEK cells and kinetics of the currents identified are similar to those noted in our study (Launay et al., 2002; Amarouch et al., 2013). Specifically, the reversal potential were found in two different studies to be around 0 mV for the transient receptor potential M4, which can form NSCCs, (Launay et al., 2002; Amarouch et al., 2013), and inward rectification was noted (Launay et al., 2002). While our data are consistent with activation of a NSCC, a caveat is that we did not definitively identify this conductance. Further, we did not explore the conductance activated when we saw outward currents in a minority of cells. Future experiments will be needed to determine whether a NSCC is involved in inward currents detected in 5-HT$_{2A}$ receptor transfected HEK cells, and to identify the conductance underlying outward currents activated in the minority of cells.

While elicitation of membrane currents in HEK cells transfected with the 5-HT$_{2A}$ receptor have never been shown, there was promising evidence suggesting the potential to elicit membrane effects with DMT and ibogaine in HEK cells transfected with the 5-HT$_{2A}$ receptor. Both compounds have been shown to be agonists at the 5-HT$_{2A}$ receptor (Sweetnam et al., 1995; Callaway et al., 1999). While DMT and ibogaine do exhibit micromolar affinity to several other receptor and transporters such as TAAR1 (trace amine-associated receptor), NMDA glutamate receptors, the serotonin, dopamine and norepinephrine transporters, alpha1-adrenergic receptors, sigma-1 receptors, muscarinic receptors-1 and −2, and κ- and μ-opioid receptors (Sweetnam et al., 1995; Su et al., 2009; Rickli et al., 2016; Glick et al., 1999; Fontanilla et al., 2009; Cozzi et al., 2009; Carbonaro and Gatch, 2016), none of these have been found to be endogenously expressed in HEK cells. The downstream G$_q$ proteins, as well as β-arrestin are present in HEK cells and have been shown to be functionally activated (Mundell et al., 1999; Mundell and Benovic, 2000). Further, agonists of the 5-HT$_{2A}$ receptor have been shown to induce cellular responses in 5-HT$_{2A}$ transfected HEK cells including rises in calcium and increases in intracellular players known to be linked to activation of the 5-HT$_{2A}$ receptor (Schachter et al., 1997; Jensen et al., 2020). In addition, membrane currents were elicited in HEK cells transfected with another GPCR, the human cannabinoid receptor1 (hCB$_1$) (Vásquez et al., 2003), indicating that transfecting HEK

cells with GPCR receptors can couple to G-proteins mediating membrane responses.

Many genetic variants of the 5-HT_{2A} receptor have been detected which could play a significant role in disease and treatment. Notably, the 5-HT_{2A}R gene exhibits significant genetic variation among individuals and several studies report an increased expression in post-mortem brains and blood platelets of depressed teenagers and adults (Pandey et al., 2002; Stanley and Mann, 1983; Mann et al., 1986; Arora and Meltzer, 1989). Genetic variability could explain differences in therapeutic outcomes and side effects observed in mood-disorder patients where SSRIs are the first choice treatment (Lucae et al., 2010). Certain naturally occurring SNPs are found to have a significant altered response to antipsychotic and antidepressive medications (Harvey et al., 2003; Davies et al., 2006; Peters et al., 2004; Lin et al., 2009; McMahon et al., 2006; Lucae et al., 2010). Our choice of 5-HT_{2A} receptor variant was based on previous literature showing that the I197V mutation exhibits a differential pharmacology in response to atypical antipsychotics (Davies et al., 2006). The synthetic mutation W200A, which is in very close proximity to the naturally occurring I197V mutation, has been shown to greatly diminish potency and efficacy for 4-methoxy-2,5-dimethoxyphenylisopropylamine (DOM) and serotonin (Harvey et al., 2003). Further, HEK cells transiently transfected with the I197V mutation has shown alterations in the relative E_{max} to 5-HT_{2A} receptor agonists with a 29 and 22 % lower relative E_{max} for 5-MeO-DMT and tryptamine, respectively (Davies et al., 2006). We noted a genotype difference in the duration of inward current induced by DMT that could be due to differences in E_{max} between the WT and I197V mutation. A lower E_{max} in the I197V variant could be caused by desensitization, internalization, or reduced activation of downstream signaling players including G proteins and intracellular proteins necessary to activate ionic conductances in HEK cells. Whether this can be transferred to e.g. *ex vivo* experimentations in rodent brain slices or the same difference is observed in humans, remains to be revealed. Taken together, our data support other findings that the I197V mutation does affect receptor functionality for DMT when it comes to the duration of the drug response, and our data suggest the utility of our HEK cell assay to screen actions of 5-HT_{2A} receptor-based agonists at genetic variants. SNPs can influence receptor function, expression levels, and drug response. Information gained could lead to a better understanding of the functional implications of these SNPs in response to psychedelics as the response to psychedelics when managing affective disorders is highly individualized, which could reflect differences in genetics. Accordingly, evaluation of functionality of different 5-HT_{2A} receptor agonists at known genetic variants of the receptor is crucial to optimize psychedelic therapies if we are to develop safe and effective treatments for a diverse population.

Our study has several limitations. We used concentrations of DMT and ibogaine which were meant to be reflective of doses experienced by humans, however, it very difficult to reconcile doses experienced by humans with concentrations used in HEK cells. In order for the psychedelic experience to occur, the plasma concentration of DMT must be around 12–90 μg/L, which corresponds to a plasma concentration around 60–500 nM (Riba et al., 2003; Callaway et al., 1999). Thus, the concentration we used in HEK cells roughly corresponds to that in the plasma. Ibogaine's affinity at the 5-HT_{2A}R has been shown to range from 4.1–92.5 μM (Zetler et al., 1972; Repke et al., 1994; Callaway et al., 1999; Helsley et al., 1998). In rodents, a concentration of 133 μM in the brain was noted with a 10 mg/kg I.V. administration (Zetler et al., 1972; Alper, 2001). While our amplitude data suggest that both concentrations activate 5-HT_{2A} receptors, our conductance data indicate that within the 30–100 μM range, actions are differential. Our data suggest the possibility of a narrow therapeutic window for ibogaine in both genotypes. Further, while we do believe that the intracellular players required to elicit a membrane response following activation of the 5-HT_{2A} receptor are present, there are limitations to studies that can be done with HEK cells. HEK cells are a useful platform for rapid screening of drug effects, and we believe they can be useful to screen functional differences in receptor variants. However, they cannot perfectly recapitulate a human cell. Relevant to this point, we provide evidence suggestive that 5-HT_{2A}R agonism in HEK cells is linked to activity of a NSCC, and while studies have demonstrated a link between 5-HT_{2A}R agonism and activation of NSCC in mammalian medial prefrontal cortical neurons, which is involved in excitation of these cells (Stephens et al., 2018), whether this ionic effect is involved in therapeutic actions in mammals remains to be determined. Further, HEK cells are not able to model significant genetic and phenotypic differences induced by human disease (Pan et al., 2009; Capes-Davis et al., 2010). In this context, induced pluripotent stem cells (iPSCs) have been shown to be an excellent model. iPSCs can be derived from patient-specific cells and differentiated into various cell types (including neurons), thus providing a unique opportunity to model human diseases *in vitro* (Wang et al., 2023; Kikuchi et al., 2017; Wen et al., 2016; Vadodaria et al., 2018). Although the utility of iPSCs to recapitulate the genetic makeup and cellular content of the patient's disease makes them an invaluable tool, their differentiation, proliferation and maturation steps not only require extensive knowledge and experience but iPSCs are also expensive to culture and maintain, so at the current time, this technical approach is not available to the majority of labs, making HEK cells an attractive option.

5. Conclusion

To the best of our knowledge, our data are the first to show that HEK cells that have been transfected with the 5-HT_{2A} receptor can respond with membrane changes upon exposure to two classic psychedelics. Further, differences in membrane effects noted between the WT 5-HT2A and the I197V SNP version of the receptor suggest that membrane changes can be used to screen differential actions of agonists at 5-HT_{2A} receptor variants. The ability to screen membrane changes induced by 5-HT_{2A} agonists in transfected HEK cells widens the potential of this relatively simple model system for screening of potential new drug candidates for agonism of the 5-HT_{2A} receptor.

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CRediT authorship contribution statement

Jannik Nicklas Eliasen: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Uffe Kristiansen:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Kristi A. Kohlmeier:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Jannik N. Eliasen (JNE) and Kristi A. Kohlmeier (KAK) conceptualized, investigated, data curated, formally analyzed, validated and visualized the study as well as acquired funding, JNE acquired and curated data, while JNE, KAK and Uffe Kristiansen (UK) interpreted the data. JNE, KAK and UK drafted, revised, reviewed and edited the article critically. KAK and UK supervised and all three authors approve the final revision. The funding source was not involved in the study design, the collection, analysis or interpretation of the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2024.149425.

Data availability

Data will be made available on request.

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