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Biased Type 1 Cannabinoid Receptor Signaling Influences Neuronal Viability in a Cell Culture Model of Huntington Disease [S]

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ABSTRACT

Huntington disease (HD) is an inherited, autosomal dominant, neurodegenerative disorder with limited treatment options. Prior to motor symptom onset or neuronal cell loss in HD, levels of the type 1 cannabinoid receptor (CB_1) decrease in the basal ganglia. Decreasing CB_1 levels are strongly correlated with chorea and cognitive deficit. CB_1 agonists are functionally selective (biased) for divergent signaling pathways. In this study, six cannabinoids were tested for signaling bias in in vitro models of medium spiny projection neurons expressing wild-type (ST*Hdh*$^{Q7/Q7}$) or mutant huntingtin protein (ST*Hdh*$^{Q111/Q111}$). Signaling bias was assessed using the Black and Leff operational model. Relative activity [$\Delta\log R$ (τ/K_A)] and system bias ($\Delta\Delta\log R$) were calculated relative to the reference compound WIN55,212-2 for $G\alpha_{i/o}$, $G\alpha_s$, $G\alpha_q$, $G\beta\gamma$, and β-arrestin1 signaling following treatment with 2-arachidonoylglycerol (2-AG), anandamide (AEA), CP55,940, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and THC+CBD (1:1), and compared between wild-type and HD cells. The E_{max} of $G\alpha_{i/o}$-dependent extracellular signal-regulated kinase (ERK) signaling was 50% lower in HD cells compared with wild-type cells. 2-AG and AEA displayed $G\alpha_{i/o}/G\beta\gamma$ bias and normalized CB_1 protein levels and improved cell viability, whereas CP55,940 and THC displayed β-arrestin1 bias and reduced CB_1 protein levels and cell viability in HD cells. CBD was not a CB_1 agonist but inhibited THC-dependent signaling (THC+CBD). Therefore, enhancing $G\alpha_{i/o}$-biased endocannabinoid signaling may be therapeutically beneficial in HD. In contrast, cannabinoids that are β-arrestin-biased—such as THC found at high levels in modern varieties of marijuana—may be detrimental to CB_1 signaling, particularly in HD where CB_1 levels are already reduced.

Introduction

Huntington Disease. Expression of mutant huntingtin protein (mHtt) causes a myriad of molecular and cellular changes that ultimately cause progressive worsening of the symptoms of Huntington disease (HD). Early in HD progression, levels of type 1 cannabinoid receptor (CB_1) mRNA and protein decrease in medium spiny projection neurons of the caudate and putamen (Denovan-Wright and Robertson, 2000; Glass et al., 2000; Van Laere et al., 2010). CB_1 transcription is inhibited by mHtt (McCaw et al., 2004; Laprairie et al., 2013). The reduction in CB_1 and loss of CB_1 function have been shown to contribute to the cognitive, behavioral, and motor deficits of HD pathology in animal models of HD (Blázquez et al., 2011; 2015; Chiarlone et al., 2014). Furthermore, rescue of CB_1 gene expression in the striatum using viral transduction prevents the loss of excitatory synaptic markers and reduces dendritic spine loss in animal models of HD (Naydenov et al., 2014). The benefit of adeno-associated viral CB_1 delivery in HD provides strong proof for the concept of treating HD through enhancing CB_1 function. However, gene-based therapies specifically for CB_1 or other single alterations in gene expression, will probably not be used clinically for HD in the near future because of the invasive nature of delivery and because the potential adverse effects of gene therapy are still being investigated. The more effective gene-based therapies for HD will target the underlying cause of the disease,

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ABBREVIATIONS: AEA, anandamide; Akt, protein kinase B; ANOVA, analysis of variance; BRET, bioluminescence resonance energy transfer; calAM, calcein-AM; CBD, cannabidiol; CB_1, type 1 cannabinoid receptor; CP, CP55,940; CREB, cAMP response element-binding protein; CTx, *Cholera* toxin; 8-OH-DPAT, 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol; D2, type 2 dopamine receptor; ERK, extracellular signal-regulated kinase; EthD-1, ethidium homodimer-1; GABA, γ-aminobutyric acid; GFP^2, green fluorescent protein 2; HD, Huntington disease; mHtt, mutant huntingtin protein; N-CB_1, amino terminus of CB_1; O-2050, (6aR,10aR)-3-(1-methanesulfonylamino-4-hexyn-6-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; p, phosphorylated; PAMs, positive allosteric modulators; PLC, phospholipase C; Rluc, *Renilla* luciferase; THC, Δ^9-tetrahydrocannabinol; URB597, [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate; WAY-100,635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide; WIN, WIN55,212–2.

the *mHtt* gene and encoded protein, and not secondarily lost cellular components (Kumar et al., 2015). In contrast, pharmacological strategies aimed at elevating CB_1 levels and/or signaling through remaining pool of CB_1 receptors has significant therapeutic potential for the treatment and management of HD.

Pharmacological Targeting of CB_1. CB_1 is activated by cannabinoids, which are a structurally diverse group of ligands that includes endogenously occurring cannabinoids (endocannabinoids) such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), phytocannabinoids from *Cannabis sativa* such as Δ^9-tetrahydrocannabinol (THC), and synthetic cannabinoids such as CP55,940 (CP) and WIN55,212–2 (WIN) (Pertwee, 2008). Activation of CB_1 in the brain results in inhibition of neurotransmitter release from presynaptic glutamatergic and GABAergic neurons and activation of prosurvival signaling cascades such as extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) (Fernández-Ruiz, 2009). We have reported that AEA, and structurally related compounds, increase the expression of CB_1 via CB_1 through $G\alpha_{i/o}$ and $G\beta\gamma$ signaling in a cell culture model expressing normal *huntingtin* ($STHdh^{Q7/Q7}$) and cells expressing mHtt ($STHdh^{Q111/Q111}$) (Laprairie et al., 2013). Importantly, this cell culture model endogenously expresses CB_1 and other components of the endocannabinoid system. Increasing levels of CB_1 improved neuronal viability in this cell culture model (Laprairie et al., 2013), lending further support to the strategy of enhancing signaling through the pool of CB_1 that are retained in the presence of mHtt and elevating CB_1 levels in these cells despite transcriptional repression via mHtt.

Not all cannabinoids increase CB_1 levels. THC and CP treatment promote β-arrestin-dependent CB_1 internalization and reduce CB_1-dependent downstream signaling (Laprairie et al., 2014). Functional selectivity (i.e., signaling bias) describes the receptor- and ligand-dependent enhancement of certain signal transduction pathways and the simultaneous diminution of other signal transduction pathways at a single receptor (Luttrell et al., 2015). Functional selectivity occurs via a GPCR ligand that preferentially activates one effector (e.g., $G\alpha_{i/o}$) more potently and efficaciously than another (e.g., β-arrestin) through ligand-specific changes in GPCR conformation or dimerization with other GPCRs (Christopoulos, 2014). Signaling bias could be exploited for enhancement of CB_1 function in HD, at the same time limiting detrimental adverse on-target effects (Laprairie et al., 2014). Cannabinoids display signaling bias (Laprairie et al., 2014; Khajehali et al., 2015). Endocannabinoids acting at CB_1 are $G\alpha_{i/o}$-biased, whereas THC and CP are β-arrestin-biased in $STHdh^{Q7/Q7}$ cells (Laprairie et al., 2014). In this study, we wanted to determine how the bias of different classes of cannabinoid affected neuronal viability. We hypothesized that $G\alpha_{i/o}$-biased cannabinoids improve neuronal viability, whereas β-arrestin-biased cannabinoids reduce—or have no effect on—cell viability. The functional selectivity of six cannabinoids [AEA, 2-AG, THC, cannabidiol (CBD), WIN, and CP] between $G\alpha_{i/o}$-, $G\alpha_s$-, $G\alpha_q$-, $G\beta\gamma$-, and β-arrestin pathways was examined in $STHdh^{Q7/Q7}$ and $STHdh^{Q111/Q111}$ cells and compared with cannabinoid-dependent changes in ATP level, γ-aminobutyric acid (GABA) release, metabolic activity, and cell death.

Materials and Methods

Drugs. Drugs were dissolved in ethanol (THC) or DMSO [2-AG, 8-OH-DPAT ($5HT_{1A}$ agonist), AEA, CP, CBD, gallein ($G\beta\gamma$ inhibitor), haloperidol (D_2 antagonist), O-2050 (CB_1 antagonist), quinpirole (D_2 agonist), WAY-100,635 ($5HT_{1A}$ antagonist), WIN] and diluted to final solvent concentrations of 0.1%. 2-AG, AEA, CP, CBD, O-2050, and WIN were purchased from Tocris Bioscience (Bristol, UK). 8-OH-DPAT, haloperidol, quinpirole, THC, and WAY-100,635 were purchased from Sigma-Aldrich (Oakville, ON, CAN). The $G\beta\gamma$ modulator gallein was purchased from MilliporeSigma (Billerica, MA). *Pertussis* toxin (PTx) and *Cholera* toxin (CTx) (Sigma-Aldrich) were dissolved in dH_2O (50 ng/ml) and added directly to the media 24 hours prior to cannabinoid treatment. Pretreatment of cells with PTx and CTx inhibits $G\alpha_{i/o}$ and $G\alpha_s$, respectively (Milligan et al., 1989). In the case of CTx, this occurs via downregulation of $G\alpha_s$ following ADP-ribosylation (Milligan et al., 1989; McKenzie and Milligan, 1991). All experiments included a vehicle treatment control.

Cell Culture. $STHdh^{Q7/Q7}$ and $STHdh^{Q111/Q111}$ cells are derived from the conditionally immortalized striatal progenitor cells of embryonic day 14 C57BlJ/6 mice (Coriell Institute, Camden, NJ) (Trettel et al., 2000). $STHdh^{Q111/Q111}$ cells express exon 1 of the mutant human *huntingtin* gene containing 111 CAG repeats knocked into the mouse *huntingtin* locus (Trettel et al., 2000). $STHdh^{Q7/Q7}$ and $STHdh^{Q111/Q111}$ cells endogenously express CB_1 and dopamine D_2 receptor (Paoletti et al., 2008; Laprairie et al., 2014). Cells were maintained at 33°C, 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10^4 IU ml^{-1} penicillin/streptomycin, and 400 μg ml^{-1} geneticin. Cells were serum-deprived for 24 hours prior to experiments to promote differentiation (Trettel et al., 2000; Laprairie et al., 2014).

Plasmids and Transfection. Human CB_1-green fluorescent protein^2 (GFP^2) C-terminal fusion protein was generated using the pGFP^2-N3 plasmid (PerkinElmer, Waltham, MA), as described previously (Bagher et al., 2013). Human arrestin2 (β-arrestin1)-*Renilla* luciferase II (Rluc) C-terminal fusion protein was generated using the pcDNA3.1 plasmid and provided by Dr. Denis J Dupré (Dalhousie University, NS). The GFP^2-Rluc fusion construct, and Rluc plasmids have also been described (Bagher et al., 2013). The $G\alpha_q$ dominant negative mutant [Glu 209 Δ Leu, Asp 277 Δ Asn (Q209L,D277N)] pcDNA3.1 plasmid was obtained from the cDNA Resource Center (Missouri S&T, Rolla, MO) (Lauckner et al., 2005).

Cells were grown in six-well plates and transfected with 200 ng of the Rluc fusion plasmid and 400 ng of the GFP^2 fusion plasmid according to previously described protocols (Laprairie et al., 2014) using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Burlington, Canada). Transfected cells were maintained for 48 hours prior to experimentation.

BRET^2. Interactions between CB_1 and β-arrestin1 were quantified via BRET^2 (Packard BioScience Company, Meriden, CT) according to previously described methods (James et al., 2006; Laprairie et al., 2014). Bioluminescence resonance energy transfer (BRET) efficiency ($BRET_{Eff}$) was determined such that Rluc alone was used to calculate $BRET_{MIN}$ and the Rluc-GFP^2 fusion protein was used to calculate $BRET_{MAX}$ using previously described methods (James et al., 2006).

On- and In-Cell Western. On-Cell Western (LI-COR Biosciences, Lincoln, NE) analyses were completed as described previously (Laprairie et al., 2014) using primary antibody directed against N-CB_1 (1:500, cat. no. 101500; Cayman Chemical Company, Ann Arbor, MI). All experiments measuring CB_1 included an N-CB_1 blocking peptide (1:500) control, which was incubated with N-CB_1 antibody (1:500). Immunofluorescence observed with the N-CB_1 blocking peptide was subtracted from all experimental replicates. In-Cell Western (LI-COR Biosciences) analyses were conducted as described previously (Laprairie et al., 2014). Primary antibody solutions were directed against: the amino terminus of CB_1 (N-CB_1) (1:500), phosphorylated (p)ERK1/2(Tyr205/185) (1:500), ERK1/2 (1:500), pCREB(S133) (1:500), cAMP response element-binding protein (CREB) (1:500), pPLCβ3(S537) (1:500), PLCβ3 (1:1000), pAkt (S473) (1:500), protein kinase B (Akt) (1:1000), or β-actin (1:2000; Santa Cruz Biotechnology, Dallas, TX). Secondary antibody solutions were: $IR^{CW700dye}$ or $IR^{CW800dye}$ (1:500; Rockland Immunochemicals, Pottstown, PA).

ATP Quantification, γ-Aminobutyric Acid Enzyme-Linked Immunosorbent Assay, and Cell Viability Assays. The CellTiter-Glo ATP quantification assay was used according to the manufacturer's

instructions (Promega, Madison, WI). The GABA enzyme-linked immunosorbent assay was conducted according to the manufacturer's instructions for mouse cell culture media (Novatein Biosciences, Boston, MA). GABA levels were reported as ΔGABA relative to GABA in vehicle-treated cells. Viability assays [calcein-AM (cal-AM), ethidium homodimer-1 (EthD-1)] were conducted according to the manufacturer's instructions (Live/Dead Cytotoxicity Assay, Life Technologies, Burlington, Canada). Cal-AM fluorescence is an indicator of cellular esterase activity and mitochondrial respiration. Cal-AM fluorescence (460/510 nm) is reported as % esterase activity relative to vehicle-treated ST*Hdh*$^{Q7/Q7}$ cells (100%). EthD-1 fluorescence is an indicator of membrane permeability and cell death. EthD-1 fluorescence (530/620 nm) is reported as % membrane permeability relative to ST*Hdh*$^{Q7/Q7}$ cells treated with 70% methanol for 30 minutes (100%). All measurements of viability (ATP, GABA, calcein-AM, EthD-1) were made 18 hours following cannabinoid treatment.

Statistical Analyses. All experiments were conducted alongside WIN as a reference ligand. Although it is often considered ideal to choose the endogenous receptor agonist as a reference ligand (Kenakin and Christopoulos, 2013), WIN was chosen as a reference ligand for these studies because: 1) it is a widely used reference compound to study CB_1-dependent signaling (Lauckner et al., 2005); 2) it acted as an agonist in all assays with nonsignificant differences in EC_{50} observed between assays; and 3) we wanted to determine whether the two endogenous cannabinoids, AEA and 2-AG, were inherently biased either in wild-type (ST*Hdh*$^{Q7/Q7}$) or mHtt-expressing (ST*Hdh*$^{Q111/Q111}$) cells. Concentration-response curves for ERK, BRET^2 (CB_1/β-arrestin1), CREB, phospholipase C (PLC)β3, and Akt are presented as % of WIN E_{max} in ST*Hdh*$^{Q7/Q7}$ cells (Griffin et al., 2007).

Concentration-response curves were fit to nonlinear regression with variable slope (four-parameter) model to determine pEC_{50} and E_{max} (Table 1), or global nonlinear regression using the operational model (Black and Leff, 1983; Ehlert et al., 2011; Kenakin et al., 2012) (eq. 1) to estimate the transduction coefficient [log(τ/K_A)], change in transducer coefficient relative to the reference ligand (ΔlogR), and bias factor (ΔΔlogR) (Prism v. 5.0, GraphPad Software Inc., San Diego, CA), as indicated. In eq. (1) E is the response, E_{max} is the maximal response, [A] is agonist concentration, n is transducer slope, τ is agonist efficacy, and K_A is the agonist's affinity for the receptor (Kenakin et al., 2012). To obtain a global least-squares fit of the data to the operational model, n was constrained to 1 and logK_A was shared between both ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ datasets and constrained to be greater than –15 (Griffin et al., 2007; Ehlert, 2015). Relative activity (ΔlogR) was calculated in Prism as the difference between transduction coefficients [log(τ/K_A)] values for two ligands, a "test" ligand, and a reference ligand (here WIN) as measured between sample-matched replicates (Kenakin et al., 2012) (eq. 2). In eq. (3) bias factor (i.e., log bias, ΔΔlogR) is the difference between response 1 (R_1) and response 2 (R_2) (Kenakin et al., 2012). All calculations of ΔΔlogR are reported using pERK response ($G\alpha_{i/o}$) as R_1. Statistical analyses were two-way analysis of variance (ANOVA) (Prism). Post-hoc analyses were performed using the Bonferroni test. Homogeneity of variance was confirmed using the Bartlett test. The level of significance was set to $P < 0.01$ where ANOVA was used or $P < 0.05$ where nonoverlapping confidence intervals (CI) were used to determine significance. Results are reported as the mean ± S.E.M. from at least four independent experiments.

$$E = \frac{E_{max}[A]^n \tau^n}{[A]^n \tau^n + ([A] + K_A)^n} \quad (1)$$

$$\Delta logR = \log(\tau/K_A)_{Test\ compound} - log(\tau/K_A)_{Ref\ compound} \quad (2)$$

$$log\ bias = \Delta\Delta logR = \Delta\Delta\log(\tau/K_A)_{R1-R2}$$

$$= \Delta log(\tau/K_A)_{R1} - \Delta log(\tau/K_A)_{R2} \quad (3)$$

TABLE 1
pEC_{50} and E_{max} of cannabinoid ligands at CB_1 in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells
Determined using nonlinear regression analysis (four parameters) in GraphPad v. 5.0. E_{max} (%) is the maximal agonist effect relative to E_{max} for WIN in ST*Hdh*$^{Q7/Q7}$ cells for each measurement. Data are expressed as mean ± S.E.M.

		ERK Response (Gαi/o)		BRET Response (β-arrestin1)		CREB Response (Gαs)		PLCβ3 Response (Gαq)		Akt Response (Gβγ)	
		pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)
WIN	ST*Hdh*$^{Q7/Q7}$	6.3 ± 0.4	101 ± 4.0	6.2 ± 0.5	102 ± 3.6	6.7 ± 0.2	115 ± 10	6.5 ± 0.8	105 ± 5.9	6.2 ± 0.8	102 ± 6.4
	ST*Hdh*$^{Q111/Q111}$	6.3 ± 0.9	51.5 ± 4.1*	6.1 ± 0.3	102 ± 4.3	7.0 ± 0.3	105 ± 6.1	6.5 ± 0.7	102 ± 5.3	6.2 ± 0.4	102 ± 5.6
CP	ST*Hdh*$^{Q7/Q7}$	6.3 ± 0.6	88.0 ± 4.7†	7.4 ± 0.2†	128 ± 4.0†	6.6 ± 0.5	443 ± 3.6†	6.2 ± 0.4	71.8 ± 2.9†	6.2 ± 0.5	68.1 ± 2.7†
	ST*Hdh*$^{Q111/Q111}$	6.4 ± 0.5	41.4 ± 4.7*	6.9 ± 0.1†	126 ± 5.1†	6.6 ± 0.7	432 ± 5.1†	6.2 ± 0.4	70.8 ± 4.1†	6.2 ± 0.7	76.0 ± 4.2†
2-AG	ST*Hdh*$^{Q7/Q7}$	6.4 ± 0.6	75.2 ± 3.6†	6.1 ± 0.7	101 ± 2.7	N.C.	N.C.	6.3 ± 0.6	87.4 ± 4.8	6.4 ± 0.8	111 ± 5.7
	ST*Hdh*$^{Q111/Q111}$	6.3 ± 0.6	54.5 ± 3.6*	6.2 ± 0.3	96.7 ± 3.9	N.C.	N.C.	6.2 ± 0.6	87.4 ± 3.6	6.4 ± 0.7	102 ± 4.5
AEA	ST*Hdh*$^{Q7/Q7}$	6.4 ± 0.8	117 ± 5.9†	6.1 ± 0.4	76.1 ± 2.0†	N.C.	N.C.	6.4 ± 0.9	101 ± 5.1	6.6 ± 0.9	115 ± 5.2
	ST*Hdh*$^{Q111/Q111}$	6.5 ± 1.7	64.5 ± 5.9*†	6.1 ± 0.7	65.0 ± 4.6†	N.C.	N.C.	6.4 ± 0.8	90.2 ± 4.8	6.5 ± 0.4	111 ± 2.8
THC	ST*Hdh*$^{Q7/Q7}$	6.0 ± 1.0	40.3 ± 2.4†	6.4 ± 0.5	98.8 ± 3.8	N.C.	N.C.	6.5 ± 1.4	71.4 ± 6.2†	6.5 ± 1.0	48.3 ± 6.7†
	ST*Hdh*$^{Q111/Q111}$	5.5 ± 1.5	33.8 ± 4.7*†	6.4 ± 0.4	107 ± 9.8	N.C.	N.C.	6.4 ± 1.5	67.9 ± 5.2†	6.5 ± 1.5	40.1 ± 5.2†
CBD	ST*Hdh*$^{Q7/Q7}$	N.C.	N.C.	N.C.	N.C.	6.2 ± 0.6	445 ± 13.1†	N.C.	N.C.	N.C.	N.C.
	ST*Hdh*$^{Q111/Q111}$	N.C.	N.C.	N.C.	N.C.	6.4 ± 0.8	348 ± 24.1†	N.C.	N.C.	N.C.	N.C.
THC + CBD	ST*Hdh*$^{Q7/Q7}$	N.C.	N.C.	6.0 ± 0.5	90.2 ± 3.0	6.2 ± 0.9	204 ± 4.4†	6.4 ± 0.7	55.4 ± 3.3†	6.0 ± 0.5	30.3 ± 3.2†
	ST*Hdh*$^{Q111/Q111}$	5.0 ± 1.1†	17.6 ± 2.3†	5.9 ± 0.5	102 ± 3.4	6.2 ± 1.1	194 ± 2.9†	6.4 ± 1.1	51.1 ± 2.8†	6.1 ± 0.6	33.2 ± 2.7†

N.C., not converged.
*$P < 0.01$ compared with ST*Hdh*$^{Q7/Q7}$ within ligand and measurement; †$P < 0.01$ compared with WIN within cell type and measurement, as determined using two-way analysis of variance followed by Bonferroni post-hoc test ($n = 4$).

Results

Cannabinoid-Dependent Signaling in the Presence of mHtt. ST*Hdh*$^{Q7/Q7}$ (Fig. 1, A–E) and ST*Hdh*$^{Q111/Q111}$ (Fig. 1, F–J) cells were treated with 10 nM–10 μM WIN, CP, 2-AG, AEA, THC, CBD, or THC+CBD (1:1), and G$\alpha_{i/o}$- (ERK1/2), β-arrestin1, Gα_s- (CREB), Gα_q- (PLCβ3), and G$\beta\gamma$-dependent (Akt) signaling were measured. The coupling of each of these signaling pathways to CB_1 and their respective G proteins or β-arrestin1 has been tested previously (Laprairie et al., 2014) and is presented in (Supplemental Fig. 1 for a subset of cannabinoids. The agonist effects of all cannabinoids

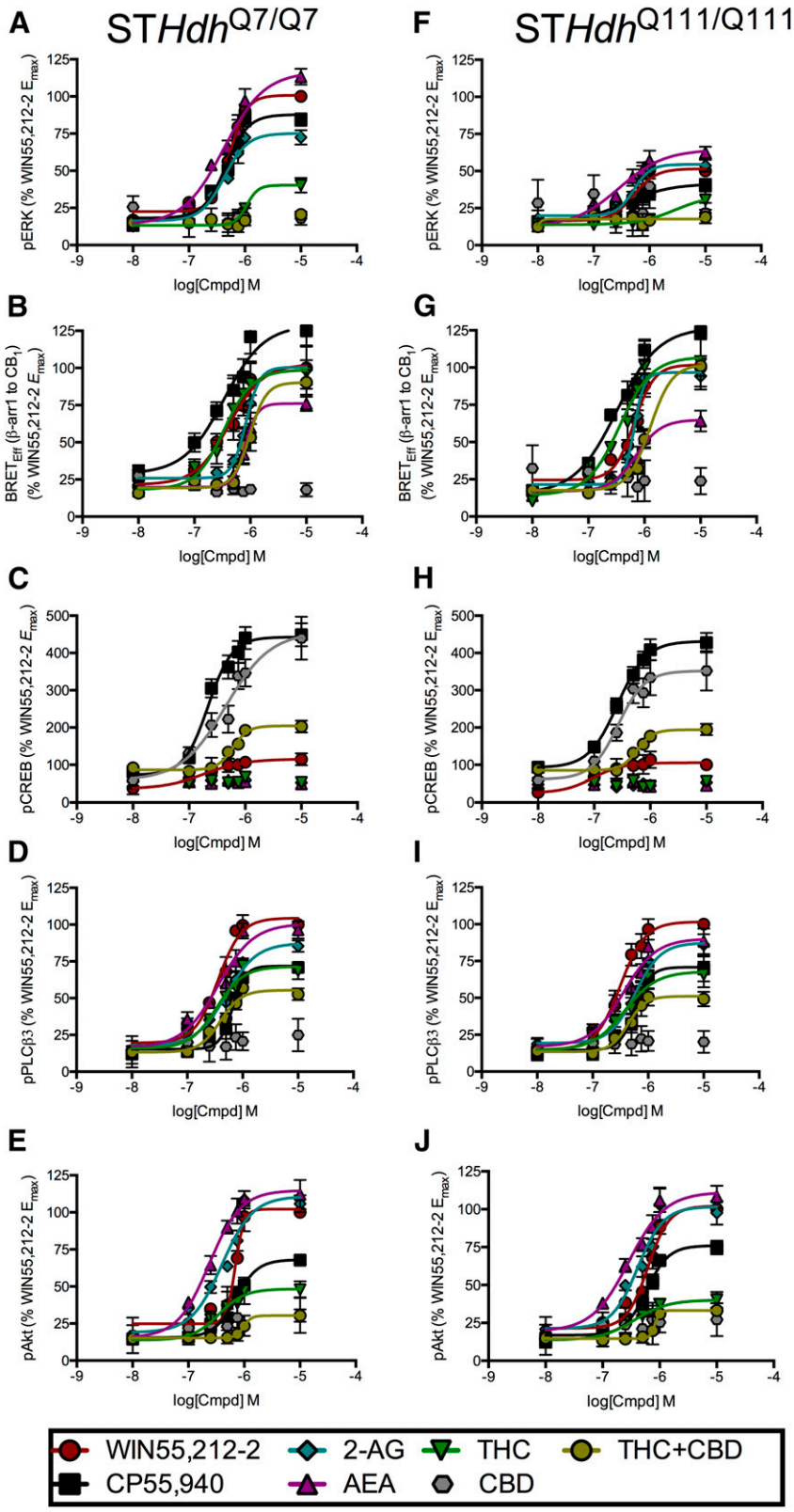

Fig. 1. Functional selectivity of cannabinoids in wild-type and mHtt-expressing cells. ST*Hdh*$^{Q7/Q7}$ (A–E) and ST*Hdh*$^{Q111/Q111}$ (F–J) cells were treated with 10–10,000 nM WIN, CP, 2-AG, AEA, THC, CBD, or THC+CBD (1:1) and ERK1/2 phosphorylation (10 minutes) (A,F), β-arrestin1 recruitment (30 minutes) (B,G), CREB phosphorylation (30 minutes) (C,H), PLCβ3 phosphorylation (10 minutes) (D,I), or Akt phosphorylation (10 minutes) (E,J) were measured and expressed relative to WIN E_{max} in ST*Hdh*$^{Q7/Q7}$ cells. ERK1/2, CREB, PLCβ3, and Akt phosphorylation were measured via in-cell Western. β-arrestin1 recruitment was measured via BRET^2. Concentration-response curves were fit to the Black-Leff global nonlinear regression using the operational model. $N = 4$.

tested were CB_1-dependent, with the exception of CBD (see below).

For pERK1/2 ($G\alpha_{i/o}$), the E_{max} observed for all cannabinoids was reduced by approximately 50% in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, with no change in pEC_{50} observed between ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (Table 1; Fig. 1, A and F). This is consistent with our earlier finding that the E_{max} for pERK relative to total ERK (i.e., raw data without reference ligand) following arachidonoyl–2′-chloroethylamide treatment is 50% lower in ST*Hdh*$^{Q111/Q111}$ cells expressing mHtt compared with ST*Hdh*$^{Q7/Q7}$ cells (Laprairie et al., 2013). The pERK E_{max} values were greater in WIN- and AEA-treated ST*Hdh*$^{Q7/Q7}$ cells compared with 2-AG-, CP-, THC-treated ST*Hdh*$^{Q7/Q7}$ cells; CBD and THC+CBD displayed no agonist activity in ST*Hdh*$^{Q7/Q7}$ cells (Table 1; Fig. 1A). In contrast, the pERK E_{max} values were not different in 2-AG-, AEA-, WIN-, and CP-treated ST*Hdh*$^{Q111/Q111}$ cells, and the pERK E_{max} was lower in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with WIN; CBD did not elicit an agonist response (Table 1; Fig. 1F). THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells also displayed a lower pEC_{50} in the pERK assay (Table 1; Fig. 1F).

CB_1 is known to interact with β-arrestin1, which mediates receptor internalization, recycling, and degradation (Sim-Selley and Martin, 2002; Laprairie et al., 2014). Unlike pERK, no differences in E_{max} and pEC_{50} were observed for β-arrestin1 assays. CP displayed higher pEC_{50} and E_{max} values than WIN, whereas no differences in pEC_{50} and E_{max} were observed between WIN, 2-AG, and THC, and AEA displayed lower E_{max} values for β-arrestin1 recruitment in both cell lines (Table 1; Fig. 1, B and G). CBD was not an agonist of β-arrestin1 recruitment. In the THC+CBD-treated cells, the E_{max} and pEC_{50} of $BRET_{Eff}$ were both reduced compared with THC-treated cells (Table 1). These data are consistent with our previous finding that CBD is a negative allosteric modulator of THC-dependent effects at CB_1 (Laprairie et al., 2015).

The observed E_{max} and pEC_{50} for pCREB ($G\alpha_S$) was not different in ST*Hdh*$^{Q7/Q7}$ cells treated with WIN, CP, CBD, or THC+CBD, relative to ST*Hdh*$^{Q111/Q111}$ cells (Table 1; Fig. 1, C and H). AEA and 2-AG did not evoke a pCREB response. CP, CBD, and THC+CBD treatment resulted in E_{max} values for pCREB higher than WIN treatment in both cell lines. pCREB pEC_{50} and E_{max} values were higher in CP- and CBD-treated cells compared with THC+CBD-treated cells (Table 1; Fig. 1, C and H). Because CB_1-dependent $G\alpha_S$ signaling is uncommon, this was examined further (see below).

CB_1 can also couple $G\alpha_q$ to modulate Ca^{2+}- and PLCβ3-dependent signaling (Lauckner et al., 2005). No differences were observed for PLCβ3 phosphorylation between ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (Table 1; Fig. 1, D and I). pPLCβ3 E_{max} values were greater in WIN-, 2-AG-, and AEA-treated cells compared with CP- and THC-treated cells, with no change in pEC_{50} (Table 1; Fig. 1, D and I). CBD was not an agonist of PLCβ3 phosphorylation.

In the case of pAkt ($G\beta\gamma$), no differences were observed between ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (Table 1; Fig. 1, E and J). pAkt E_{max} values were greater in WIN-, 2-AG-, and AEA-treated cells compared with CP-treated cells, which were in turn greater compared with THC-treated cells (Table 1; Fig. 1, E and J). pAkt pEC_{50} values did not differ between agonists. CBD was not an agonist of Akt phosphorylation.

Operational Model Analysis of Cannabinoid Transduction Coefficients (logR) and Relative Activity (ΔlogR) in the Presence of mHtt. The operational model global nonlinear regression (eq. 1) was used to analyze concentration-response data for cannabinoid signaling bias in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells. CBD only displayed agonist activity for pCREB and these data were therefore omitted from global nonlinear regression analyses of pERK, β-arrestin1, pPLCβ3, and pAkt assays. The transduction coefficient [logR (τ/K_A)] for the ERK response was lower in THC- and THC+CBD-treated cells compared with WIN-treated cells, and was lower in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells (Table 2). logR for β-arrestin1 was also lower in THC- (only ST*Hdh*$^{Q111/Q111}$) and THC+CBD-treated cells compared with WIN-treated cells, was lower in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, and was higher in THC- and THC+CBD-treated cells compared with the ERK response (Table 2). logR for the CREB response was higher in CP-treated cells, and lower in THC+CBD-treated cells, compared with WIN, was lower in WIN-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, and was lower in WIN-treated cells compared with the ERK response (Table 2, 3). logR for the PLCβ3 response was lower in CP- (only ST*Hdh*$^{Q7/Q7}$), AEA-, THC-, and THC+CBD-treated cells, compared with WIN, was lower in CP-, AEA-, and THC-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, and was lower in AEA- and THC-treated cells compared with the ERK response (Table 2, 3). Finally, logR for the Akt response was lower in CP-, THC-, and THC+CBD-treated cells, was lower in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, and was lower in THC-treated ST*Hdh*$^{Q7/Q7}$ cells compared with the ERK response (Table 2, 4).

Relative activity (ΔlogR) was calculated using WIN as the reference ligand (eq. 2). WIN was chosen as a reference ligand, rather than the endocannabinoids 2-AG and AEA (Kenakin and Christopoulos, 2013), because it displayed activity in all assays, and we wanted to quantify the relative activity and bias of 2-AG and AEA in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells. The ΔlogR for ERK response was lower in THC- and THC+CBD-treated cells compared with WIN (ΔlogR = 0) (Table 2). The ΔlogR for β-arrestin1 was lower in 2-AG-, AEA-, THC-, and THC+CBD-treated cells compared with WIN, and compared with the ERK response (Table 2). The ΔlogR for β-arrestin1 was lower in THC-treated ST*Hdh*$^{Q111/Q111}$ cells, and higher in THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells, compared with ST*Hdh*$^{Q7/Q7}$ cells (Table 2). The ΔlogR for the CREB response was higher in CP- (both cell types) and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells, and lower in THC+CBD-treated ST*Hdh*$^{Q7/Q7}$ cells, compared with WIN (Table 3). The ΔlogR for the CREB response was higher in CP- (both cell types) and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with the ERK response, and was greater in THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells (Table 2, 3). The ΔlogR for the PLCβ3 response was lower in CP- (only ST*Hdh*$^{Q7/Q7}$), 2-AG- (only ST*Hdh*$^{Q7/Q7}$), AEA- (only ST*Hdh*$^{Q111/Q111}$), THC- and THC+CBD-treated cells compared with WIN, and compared with the ERK response for CP, 2-AG, and AEA treatments (Table 2, 3). The ΔlogR for the PLCβ3 response was lower in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells compared with

TABLE 2

Transduction coefficients and relative activity of cannabinoid ligands at CB_1 in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells: ERK and BRET Responses

Determined using the operational model global nonlinear regression analysis (eqs. 1 and 2) in GraphPad v. 5.0. Data are expressed as mean with 95% confidence interval.

		ERK Response (Gαi/o)				BRET Response (β-arrestin1)			
		logR (τ/K_A)		ΔlogR (τ/K_A)[a]		logR (τ/K_A)		ΔlogR (τ/K_A)[a]	
WIN	ST*Hdh*$^{Q7/Q7}$	6.35	(6.33–6.37)	Reference ligand		6.41	(6.36–6.46)	Reference ligand	
	ST*Hdh*$^{Q111/Q111}$	6.33	(6.28–6.38)	Reference ligand		6.41	(6.38–6.44)	Reference ligand	
CP	ST*Hdh*$^{Q7/Q7}$	6.30	(6.26–6.34)	−0.04	(–0.09–0.01)	6.46	(6.41–6.52)	0.05	(–0.03–0.13)
	ST*Hdh*$^{Q111/Q111}$	6.22	(6.17–6.27)	−0.11	(–0.22–0.02)	6.47	(6.42–6.49)	0.06	(–0.01–0.11)
2-AG	ST*Hdh*$^{Q7/Q7}$	6.28	(6.20–6.36)	−0.07	(–0.14–0.00)	6.15	(5.91–6.37)	−0.23	(–0.24–0.22)*†
	ST*Hdh*$^{Q111/Q111}$	6.28	(6.21–6.35)	−0.05	(–0.11–0.01)	6.27	(6.36–6.38)	−0.13	(–0.23–0.03)*
AEA	ST*Hdh*$^{Q7/Q7}$	6.35	(6.34–6.36)	0.00	(–0.01–0.01)	6.09	(5.82–6.37)	−0.31	(–0.33–0.29)*†
	ST*Hdh*$^{Q111/Q111}$	6.42	(6.36–6.48)	0.09	(–0.02–0.20)	6.22	(6.07–6.37)	−0.18	(–0.26–0.10)*†
THC	ST*Hdh*$^{Q7/Q7}$	4.48	(4.43–4.54)*	−1.83	(–2.97–0.69)*	6.41	(6.40–6.42)†	0.00	(–0.01–0.01)†
	ST*Hdh*$^{Q111/Q111}$	3.26	(3.22–3.30)*^	−3.01	(–4.43–1.59)*	4.98	(4.94–5.02)*^†	−1.43	(–1.47–1.39)*^†
CBD	ST*Hdh*$^{Q7/Q7}$		N.C.		N.C.		N.C.		N.C.
	ST*Hdh*$^{Q111/Q111}$		N.C.		N.C.		N.C.		N.C.
THC + CBD	ST*Hdh*$^{Q7/Q7}$	2.06	(1.91–2.21)*	−4.29	(–5.95–2.63)*	0.83	(–1.91–1.95)*†	−5.58	(–5.60–5.56)*
	ST*Hdh*$^{Q111/Q111}$	0.35	(–2.01–3.69)*^	−1.40	(–2.77–0.33)*	4.83	(4.77–4.89)*^†	−1.58	(–1.64–1.52)*^†

N.C., not converged.

*$P < 0.05$ compared with WIN within cell type and measurement; ^$P < 0.05$ compared with ST*Hdh*$^{Q7/Q7}$ within ligand and measurement; †$P < 0.05$ compared with ERK (G$\alpha_{i/o}$) within cell type, as determined using nonoverlapping confidence intervals ($n = 4$).

[a]ΔlogR (τ/K_A) calculated as logR (τ/K_A) test ligand – logR (τ/K_A) reference ligand within cell type, where **WIN** is the reference ligand and ΔlogR (τ/K_A) 'WIN' = 0.

ST*Hdh*$^{Q7/Q7}$ cells (Table 2, 3). Finally, the ΔlogR for the Akt response was lower in CP- (only ST*Hdh*$^{Q7/Q7}$), AEA- (only ST*Hdh*$^{Q7/Q7}$), THC-, and THC+CBD-treated cells compared with WIN, and compared with the ERK response for CP and THC (Table 2, 4). ΔlogR values for the Akt response were lower and higher in THC- and THC+CBD-treated ST*Hdh*$^{Q111/Q111}$ cells, respectively, compared with ST*Hdh*$^{Q7/Q7}$ cells (Table 2, 4).

Summarizing the data in Table 2, we observed that the rank order of τ/K_A and relative activity (ΔlogR) for pERK was AEA > WIN > CP (ST*Hdh*$^{Q7/Q7}$) > 2-AG > CP (ST*Hdh*$^{Q111/Q111}$) > THC ≥ THC+CBD. For β-arrestin1 this order was CP > THC ≥ WIN > 2-AG = AEA > THC (ST*Hdh*$^{Q111/Q111}$) > THC+CBD. For pCREB this order was CP > WIN (ST*Hdh*$^{Q7/Q7}$) > CBD (ST*Hdh*$^{Q7/Q7}$) > THC+CBD (ST*Hdh*$^{Q111/Q111}$) > CBD (ST*Hdh*$^{Q111/Q111}$) > WIN (ST*Hdh*$^{Q111/Q111}$) ≥ THC+CBD (ST*Hdh*$^{Q7/Q7}$). For pPLCβ3 the order was WIN > CP (ST*Hdh*$^{Q111/Q111}$) > AEA (ST*Hdh*$^{Q7/Q7}$) > 2-AG (ST*Hdh*$^{Q7/Q7}$) > CP (ST*Hdh*$^{Q7/Q7}$) > 2-AG (ST*Hdh*$^{Q7/Q7}$) > THC (ST*Hdh*$^{Q7/Q7}$) > AEA (ST*Hdh*$^{Q7/Q7}$) > THC (ST*Hdh*$^{Q7/Q7}$) > THC+CBD. And for pAkt the order was AEA ≥ 2-AG = WIN > CP > THC > THC+CBD.

Operational Model Analysis of Cannabinoid-Dependent System Bias ($\Delta\Delta$logR) in the Presence of mHtt. Bias values ($\Delta\Delta$logR) were calculated from the relative activity data (ΔlogR) to characterize functional selectivity in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (eq. 3) (Fig. 2, A–D). Because CB_1 is classically considered a G$\alpha_{i/o}$-coupled receptor (Kondo et al., 1998; Lauckner et al., 2005), all comparisons were made using G$\alpha_{i/o}$-dependent ERK1/2 signaling (pERK) as $\Delta\log R_1$. On the basis of these data, CP evoked Gα_S- and β-arrestin1-biased signaling compared with G$\alpha_{i/o}$, and G$\alpha_{i/o}$-biased signaling compared with Gα_q or G$\beta\gamma$ in both cell types tested here (i.e., Gα_S > β-arrestin1 > G$\alpha_{i/o}$ > Gα_q > G$\beta\gamma$) (Fig. 2, A–D). 2-AG evoked G$\alpha_{i/o}$-biased signaling compared with β-arrestin1 (in ST*Hdh*$^{Q7/Q7}$ cells) and Gα_q (more so in ST*Hdh*$^{Q111/Q111}$ cells), and G$\beta\gamma$-biased signaling compared with G$\alpha_{i/o}$ (in ST*Hdh*$^{Q7/Q7}$ cells) (i.e., G$\beta\gamma$ > G$\alpha_{i/o}$ > β-arrestin1 > Gα_q) (Fig. 2, A–D). Like 2-AG, AEA evoked G$\alpha_{i/o}$-biased signaling compared with β-arrestin1 and Gα_q (more so in ST*Hdh*$^{Q111/Q111}$ cells), and G$\beta\gamma$-biased signaling compared with G$\alpha_{i/o}$ (in ST*Hdh*$^{Q7/Q7}$ cells) (i.e., G$\beta\gamma$ > G$\alpha_{i/o}$ > β-arrestin1 > Gα_q) (Fig. 2, A–D). THC evoked β-arrestin1-, Gα_q-, and G$\beta\gamma$-biased signaling compared with G$\alpha_{i/o}$, in both

TABLE 3

(*Continued from Table 2:* CREB and PLCβ3 Responses)

		CREB Response (Gαs)				PLCβ3 Response (Gαq)			
		logR (τ/K_A)		ΔlogR (τ/K_A)[a]		logR (τ/K_A)		ΔlogR (τ/K_A)[a]	
WIN	ST*Hdh*$^{Q7/Q7}$	3.43	(3.32–3.54)†	Reference ligand		6.54	(6.32–6.72)	Reference ligand	
	ST*Hdh*$^{Q111/Q111}$	2.22	(2.20–2.24)^†	Reference ligand		6.51	(6.34–6.66)	Reference ligand	
CP	ST*Hdh*$^{Q7/Q7}$	6.47	(6.46–6.48)*	3.01	(2.91–3.11)*†	5.77	(5. 67–5.87)*†	−0.77	(–0.92–0.62)*†
	ST*Hdh*$^{Q111/Q111}$	5.07	(5.06–5.08)*	2.85	(2.55–3.04)*†	6.32	(4.35–8.29)^	−0.21	(–0.48–0.06)^
2-AG	ST*Hdh*$^{Q7/Q7}$		N.C.		N.C.	6.01	(4.66–7.36)	−0.53	(–0.88–0.18)*†
	ST*Hdh*$^{Q111/Q111}$		N.C.		N.C.	5.76	(4.99–6.53)	−0.71	(–1.46–0.04)
AEA	ST*Hdh*$^{Q7/Q7}$		N.C.		N.C.	6.31	(6.08–6.54)	−0.23	(–0.47–0.01)
	ST*Hdh*$^{Q111/Q111}$		N.C.		N.C.	5.41	(4.52–5.94)*^†	−1.13	(–1.99–0.27)*†
THC	ST*Hdh*$^{Q7/Q7}$		N.C.		N.C.	5.45	(5.23–5.67)*†	−1.09	(–1.31–0.87)*
	ST*Hdh*$^{Q111/Q111}$		N.C.		N.C.	4.33	(3.80–4.86)*^†	−2.18	(–2.69–1.67)*^
CBD	ST*Hdh*$^{Q7/Q7}$	3.34	(3.29–3.39)	−0.09	(–0.22–0.04)		N.C.		N.C.
	ST*Hdh*$^{Q111/Q111}$	2.27	(2.24–2.30)	0.03	(–0.01–0.07)		N.C.		N.C.
THC + CBD	ST*Hdh*$^{Q7/Q7}$	0.26	(–0.40–1.92)*	−3.19	(–3.21–3.17)*	0.57	(–1.43–1.91)*	−5.97	(–6.20–5.74)*
	ST*Hdh*$^{Q111/Q111}$	3.28	(3.27–3.30)*†	1.06	(1.04–1.08)*^†	4.25	(3.55–4.95)*^	−2.27	(–2.95–1.59)*^

TABLE 4
(*Continued from Table 2:* Akt Response)

		Akt Response (G$\beta\gamma$)			
		logR (τ/K_A)		ΔlogR (τ/K_A)[a]	
WIN	ST*Hdh*$^{Q7/Q7}$	6.18	(5.98–6.40)	Reference ligand	
	ST*Hdh*$^{Q111/Q111}$	6.21	(6.13–6.29)	Reference ligand	
CP	ST*Hdh*$^{Q7/Q7}$	5.94	(5.92–5.96)*†	−0.24	(–0.26–0.22)*†
	ST*Hdh*$^{Q111/Q111}$	5.84	(5.31–6.37)	−0.37	(–0.91–0.17)
2-AG	ST*Hdh*$^{Q7/Q7}$	6.22	(6.19–6.25)	0.02	(–0.01–0.05)
	ST*Hdh*$^{Q111/Q111}$	6.14	(5.96–6.32)	−0.07	(–0.25–0.11)
AEA	ST*Hdh*$^{Q7/Q7}$	6.32	(6.27–6.37)	0.14	(–0.03–0.25)*
	ST*Hdh*$^{Q111/Q111}$	6.25	(5.73–6.77)	0.04	(–0.47–0.55)
THC	ST*Hdh*$^{Q7/Q7}$	5.35	(5.32–5.38)*†	−0.83	(–0.86–0.80)*†
	ST*Hdh*$^{Q111/Q111}$	4.00	(3.87–4.13)*^	−2.21	(–2.32–2.10)*^†
CBD	ST*Hdh*$^{Q7/Q7}$		N.C.		N.C.
	ST*Hdh*$^{Q111/Q111}$		N.C.		N.C.
THC + CBD	ST*Hdh*$^{Q7/Q7}$	0.31	(–1.39–2.01)*†	−5.87	(–5.97–5.77)*
	ST*Hdh*$^{Q111/Q111}$	3.59	(3.50–3.68)*^	−2.62	(–2.72–2.52)*^

cell types (i.e., β-arrestin1 > Gα_q = G$\beta\gamma$ > G$\alpha_{i/o}$) (Fig. 2, A–D). CBD treatment only produced a significant activation of Gα_S-dependent CREB phosphorylation, and bias values could not be calculated for this ligand. The combination THC+CBD evoked Gα_S-biased signaling compared with G$\alpha_{i/o}$- and G$\alpha_{i/o}$-biased signaling compared with β-arrestin1, Gα_q, or G$\beta\gamma$ (more so in ST*Hdh*$^{Q7/Q7}$ cells) (i.e., Gα_S > G$\alpha_{i/o}$ > β-arrestin1 = Gα_q = G$\beta\gamma$) (Fig. 2, A–D).

Each cannabinoid analyzed here displayed unique functional selectivity for different signaling pathways. Overall, the bias factor of 2-AG and AEA was shifted toward G$\alpha_{i/o}$-dependent ERK phosphorylation, and the bias factor of THC+CBD was shifted away from G$\alpha_{i/o}$-dependent ERK phosphorylation, in ST*Hdh*$^{Q111/Q111}$ cells. The reduced pERK E_{max} in mHtt-expressing ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells (Table 1) may result from lower CB_1 levels (50%) (Laprairie et al., 2013). An important advantage of using the operational model to estimate the relative activity and ligand bias is that this model negates the effects of differences in receptor density (Kenakin et al., 2012). Therefore, differences in bias between ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells were probably mHtt-dependent and not the result of changes in agonist potency or efficacy.

Cannabinoid-Specific Changes in Cellular Function and Viability. Treatment of ST*Hdh*$^{Q7/Q7}$ cells with WIN, 2-AG, AEA, or THC resulted in a small increase in ATP, whereas treatment with CP, CBD, or THC+CBD resulted in a decrease in ATP (Fig. 3A). In ST*Hdh*$^{Q111/Q111}$ cells, basal ATP levels were approximately 50% lower than basal ATP levels in ST*Hdh*$^{Q7/Q7}$ cells. ATP levels increased in ST*Hdh*$^{Q111/Q111}$ cells treated with WIN, 2-AG, AEA, or THC and decreased with CP or CBD (Fig. 3E). THC+CBD treatment resulted in higher ATP levels in ST*Hdh*$^{Q111/Q111}$ cells. CP and CBD were the only cannabinoids tested that evoked Gα_S-biased (CREB) signaling in ST*Hdh* cells. The lower ATP levels observed in cells treated with CP or CBD may have resulted from cAMP production. However, given that cells expressing mHtt are deficient in ATP (Sadri-Vakili et al., 2006; Laprairie et al., 2013), cannabinoids that exaggerate this state may exacerbate cellular pathology.

Excessive glutamate release from cortical neurons and GABA release from striatal medium spiny projection neurons are both observed in HD (Benn et al., 2007; Botelho et al., 2014). Compounds that limit neurotransmitter release may, therefore, be beneficial in HD, whereas compounds that enhance neurotransmitter release may exacerbate HD pathophysiology. GABA release was inhibited by WIN, 2-AG, AEA, CP, and THC in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (Fig. 3, B and F). CBD treatment was associated with enhanced GABA release in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells and the EC_{50} and E_{max} of this response were reduced in the presence of THC (THC+CBD) (Fig. 3, B and F). Therefore, CBD treatment may enhance excessive neurotransmitter release in HD, whereas other cannabinoids tested here limited neurotransmitter release.

Cell viability was measured by cal-AM fluorescence, which is an indicator of esterase activity and mitochondrial respiration that is positively correlated with viability, and EthD-1 fluorescence, which is an indicator of membrane permeability and cell death and therefore negatively correlated with viability (MacCoubrey et al., 1990). Basal cal-AM fluorescence (% esterase activity) was 60% less in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells (Fig. 3, C and G). Cal-AM fluorescence was decreased by 40% in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells treated with CP or THC and increased by 40% in ST*Hdh*$^{Q111/Q111}$ cells treated with WIN, 2-AG, AEA, or CBD (Fig. 3, C and G). Basal EthD-1 fluorescence (% membrane permeable cells) was 40% greater in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells (Fig. 3, D and H). EthD-1 fluorescence was increased by 30% in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells treated with CP or THC (Fig. 3, D and H). EthD-1 fluorescence was decreased by 20% in AEA- and CBD-treated ST*Hdh*$^{Q7/Q7}$ cells, and by 40% in WIN-, 2-AG-, AEA-, and CBD-treated ST*Hdh*$^{Q111/Q111}$ cells (Fig. 3, D and H). The effect of CBD predominated over that of THC for both cal-AM and EthD-1 fluorescence in both cell lines. Therefore, in these viability assays, the CP and THC (which both displayed β-arrestin1 bias) appeared harmful, whereas other cannabinoids improved viability in ST*Hdh*$^{Q111/Q111}$ cells.

Functional CB_1 residing at the plasma membrane undergo internalization following ligand binding and β-arrestin recruitment (Blair et al., 2009). Total CB_1 levels were higher in WIN-, 2-AG-, and AEA-treated ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells, compared with vehicle, whereas total CB_1 levels were lower in CP- and THC-treated ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (Fig. 4A). The fraction of CB_1 at the

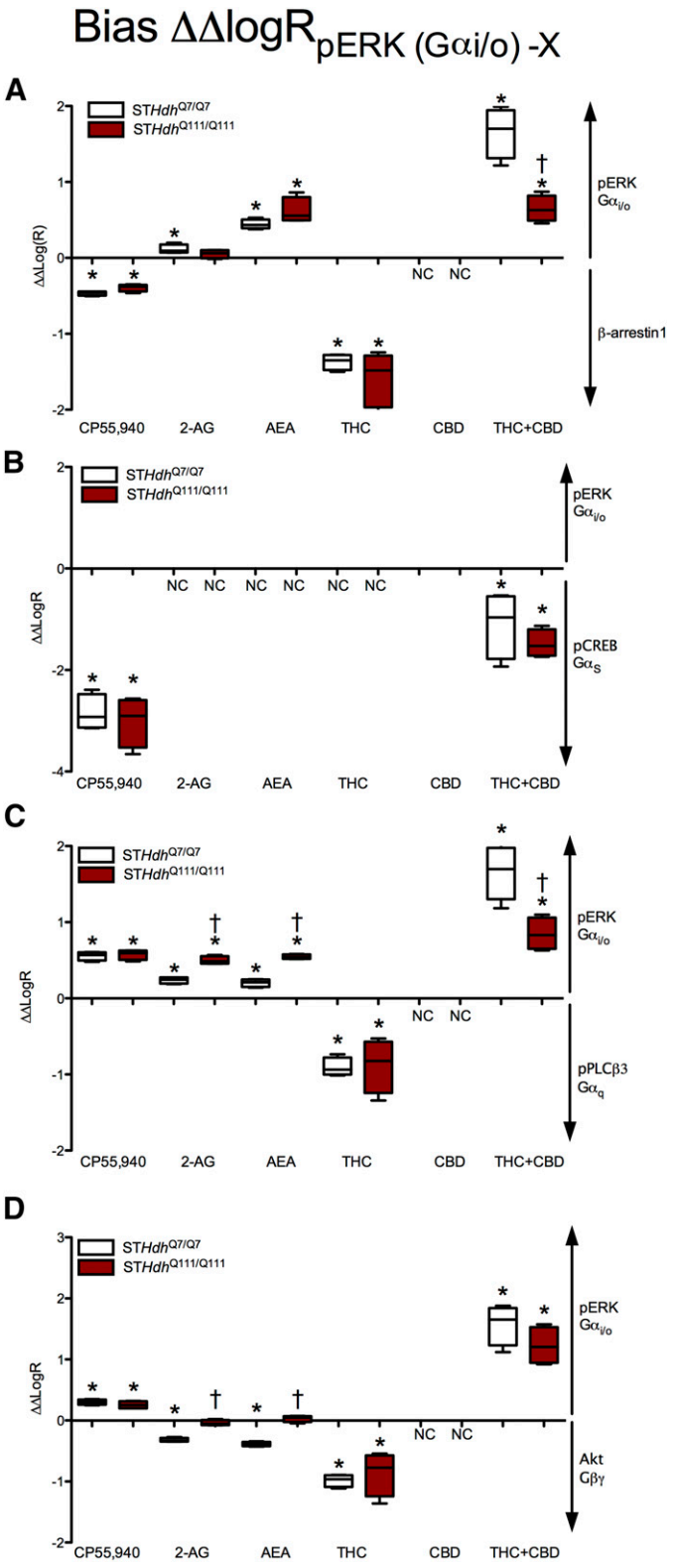

Fig. 2. Calculated bias factor of cannabinoids in wild-type and mHtt-expressing cells. Ligand bias (ΔΔlogR) was calculated using eq. (2) as the difference between the ERK ($G\alpha_{i/o}$) response and a second response X: (A) β-arrestin1, (B) $G\alpha_s$, (C) $G\alpha_q$, or (D) $G\beta\gamma$. Data are displayed as the mean with the minimum and maximum (box) and 95% confidence intervals (error bars). $^*P < 0.01$ compared with 0 (i.e., no bias), $\dagger P < 0.01$ compared with ST*Hdh*$^{Q7/Q7}$ cells within ligand. $N = 4$.

plasma membrane and total CB_1 was assayed in ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells treated with various cannabinoids for 12 hours (Fig. 4, A and B). The fraction of CB_1 at the plasma membrane was lower in WIN-, 2-AG-, CP-, and THC-treated cells, and higher in CBD-treated cells (Fig. 4B). CP and THC—and to a lesser extent WIN and 2-AG—displayed greater β-arrestin1 bias than AEA or CBD. The mechanism of cannabinoid-dependent induction of CB_1 expression has been described previously (Laprairie et al., 2013). Here, it is important to note that treatment with cannabinoids that evoked $G\alpha_{i/o}$-and $G\beta\gamma$-biased signaling (2-AG, AEA) was associated with higher CB_1 levels, whereas treatment with CP and THC (β-arrestin1-biased cannabinoids) was associated with lower CB_1 levels, suggesting that cannabinoids that are functionally selective for β-arrestin1 may reduce the available pool of CB_1 receptors. The effects of THC and CBD were neutralized by one another (Fig. 4, A and B).

Mechanism of CP- and CBD-Dependent $G\alpha_S$ Signaling. CBD is known to modulate the activity of many cellular GPCRs, including CB_1, the type 2 cannabinoid receptor (CB_2) (Hayakawa et al., 2008), the serotonin $5HT_{1A}$ receptor (Russo et al., 2005), G protein-coupled receptor 55(Ryberg et al., 2007), and the μ- and δ-opioid receptors (Kathmann et al., 2006). Here, CBD treatment resulted in CB_1-independent CREB phosphorylation (Fig. 5). CREB phosphorylation was highest 30 minutes after CBD treatment and was sustained for the duration of the experiment (60 minutes) (Fig. 5A). Treatment of ST*Hdh*$^{Q7/Q7}$ cells with the $5HT_{1A}$ agonist 8-OH-DPAT resulted in a dose-dependent increase in CREB phosphorylation that was competitively inhibited by the $5HT_{1A}$ antagonist WAY-100,635 and CBD (Fig. 5B). Treatment of ST*Hdh*$^{Q7/Q7}$ cells with CBD alone also resulted in a dose-dependent increase in CREB phosphorylation, with less potency and efficacy that the full agonist 8-OH-DPAT (Fig. 5C). CBD-dependent CREB phosphorylation was not inhibited by the CB_1 antagonist O-2050, but was inhibited by WAY-100,635 (Fig. 5C), indicating that CBD activated CREB via $5HT_{1A}$. It is not known whether the partial agonism of $5HT_{1A}$ by CBD is functionally antagonistic of serotonergic signaling in vivo and whether this would play a role in CBD-based treatments of neurologic disorders.

Unexpectedly, we observed a switch in signaling following continued drug exposure for CP. At 10 minutes CP treatment produced $G\alpha_{i/o}$-dependent ERK phosphorylation that returned to basal levels by 25 minutes; and at 30 minutes CP treatment produced $G\alpha_s$-dependent CREB phosphorylation (Fig. 5A). ST*Hdh* cells endogenously express the type 2 dopamine receptor (D_2) (Paoletti et al., 2008) and heterodimerization of CB_1 and D_2 is known to lead to a switch in coupling from $G\alpha_{i/o}$ to $G\alpha_s$ following treatment with CP (Glass and Felder, 1997; Kearn et al., 2005). Therefore, we hypothesized that CP could be functionally selective for CB_1/D_2 heterodimer signaling to explain the switch from $G\alpha_{i/o}$ to $G\alpha_s$. Cotreatment of ST*Hdh*$^{Q7/Q7}$ cells with CP and 1 μM quinpirole (a D_2 agonist) shifted the concentration-response curve for CREB phosphorylation right, as did cotreatment with O-2050 (a competitive antagonist of CB_1), whereas cotreatment with 10 μM haloperidol (a D_2 antagonist) shifted the concentration-response curve left (Fig. 5D). Quinpirole and haloperidol did not effect CREB phosphorylation alone (Fig. 5D). From these data, we suggest that CP selectively enhanced either physical heterodimerization between CB_1/D_2

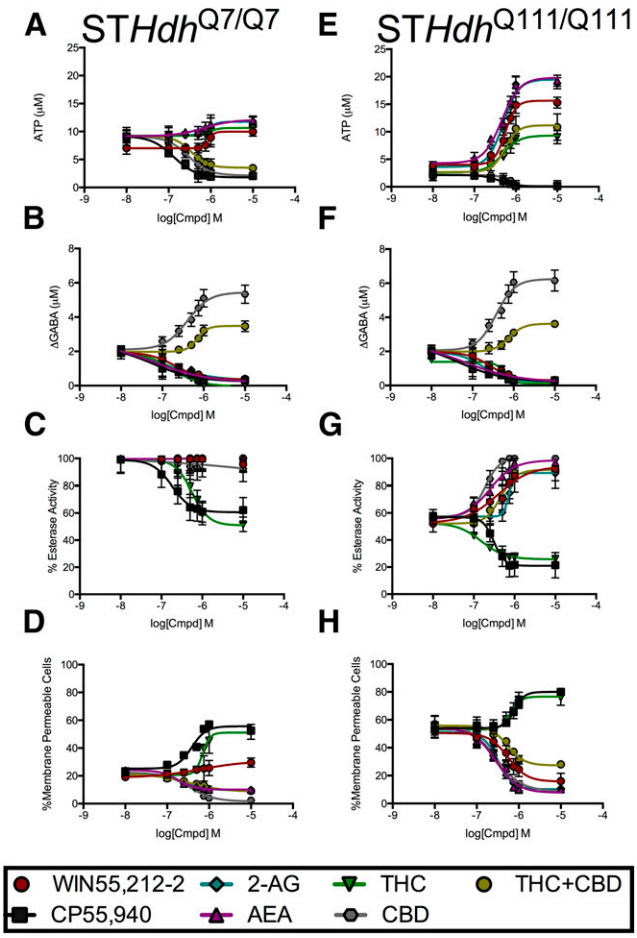

Fig. 3. Changes in functionality and viability in wild-type and mHtt-expressing cells treated with cannabinoids. ST*Hdh*$^{Q7/Q7}$ (A–D) and ST*Hdh*$^{Q111/Q111}$ (E–H) cells were treated with 10–10,000 nM WIN, CP, 2-AG, AEA, THC, CBD, or THC+CBD (1:1) for 30 minutes, and ATP (A,E), change in GABA release compared with vehicle treatment (ΔGABA) (B,F), % cellular esterase activity compared with vehicle treatment (C,G), and % membrane-permeable cells compared with vehicle treatment (D,H) were measured. [ATP] was determined using the CellTiter Glo assay (Promega). [GABA] in cell culture media was determined using GABA enzyme-linked immunosorbent assay (Novatein Bio, Woburn MA). % Cellular esterase activity (calcein-AM cleavage) and % membrane permeable cells (ethidium homodimer-1 penetration) were determined using the Live/Dead cytotoxicity assay (Invitrogen/ThermoFisher Scientific, Grand Island, NY). Concentration-response curves were fit using nonlinear regression models. $N = 4$.

or functional signaling through these receptors with a subsequent switch from $G\alpha_{i/o}$ *to* $G\alpha_s$ (Kearn et al., 2005).

Discussion

Correlations between Functional Selectivity and Cellular Viability. In this study, we described the biased signaling properties of six cannabinoids in the ST*Hdh* cell culture model of striatal medium spiny projection neurons. System bias shifted toward $G\alpha_{i/o}$ for 2-AG and AEA in ST*Hdh*$^{Q111/Q111}$ (mHtt-expressing) cells compared with ST*Hdh*$^{Q7/Q7}$ cells. Treatment of ST*Hdh*$^{Q111/Q111}$ cells with cannabinoids that signaled via CB_1 and were functionally selective for $G\alpha_{i/o}$ and $G\beta\gamma$ (2-AG, AEA) was associated with the greatest improvement in ATP production, inhibition of GABA release, cellular metabolic activity (esterase activity), and cell death (membrane permeability). In contrast, ligands that preferentially enhanced β-arrestin1-recruitment (THC and CP) reduced cellular viability in both ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells as determined by the same measures. We have previously observed that derivatives of AEA normalize CB_1 levels in ST*Hdh*$^{Q111/Q111}$ cells via $G\alpha_{i/o}$, $G\beta\gamma$, Akt, and nuclear factor (NF)-κB, and that normalization of CB_1 was associated with improved cell function and viability (Laprairie et al., 2013, 2014). Recently, three studies have demonstrated that increasing CB_1 levels in medium spiny projection neurons in the R6/2 mouse model of HD via adenovirus-mediated overexpression normalizes brain-derived neurotrophic factor levels, reduces striatal atrophy, and prevents decreases in dendritic spine density and levels of excitatory synaptic markers, such as synaptophysin and vesicular glutamate transporter, but does not improve deficits in motor coordination (Chiarlone et al., 2014; Naydenov et al., 2014; Blázquez et al., 2015). In accordance with this, knockdown or knockout of CB_1 in medium spiny projection neurons of R6/2, N171-82Q, or *Hdh*$^{Q150/Q150}$ HD mice further reduces the pool of CB_1 and exacerbates deficits in motor control, enhances striatal atrophy, and reduces survival (Blázquez et al., 2011; Mievis et al., 2011; Horne et al., 2013). Further, individuals with HD and a variant of the CB_1 gene (*CNR1* rs4707436) that is associated with lower levels of CB_1 begin displaying motor-related symptoms of HD earlier than individuals with HD and normal *CNR1* (Kloster et al., 2013). Together, these studies and our

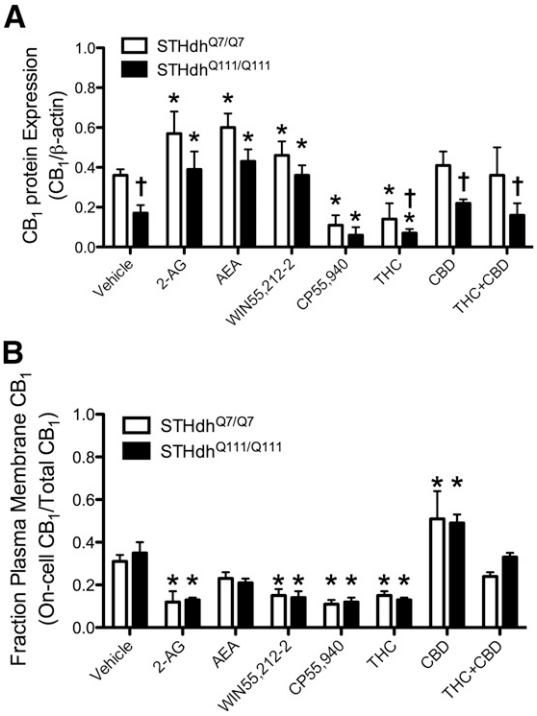

Fig. 4. Long-term exposure to cannabinoids affected CB_1 localization and levels. ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells were treated with 1.0 μM 2-AG, AEA, WIN, CP, THC, CBD, or THC+CBD (1:1) for 12 hours, and total CB_1 levels (A) and the fraction of CB_1 at the plasma membrane (B). (A) Total CB_1 levels were determined using In-Cell Western and expressed relative to β-actin levels. $N = 8$. (B) The fraction of CB_1 at the plasma membrane was determined using On- and In-Cell Western. $N = 8$. $^*P < 0.01$ compared with vehicle-treated cells within cell type, $^\dagger P < 0.01$ compared with ST*Hdh*$^{Q7/Q7}$ cells within treatment group, as determined using two-way ANOVA followed by Bonferroni post-hoc analysis.

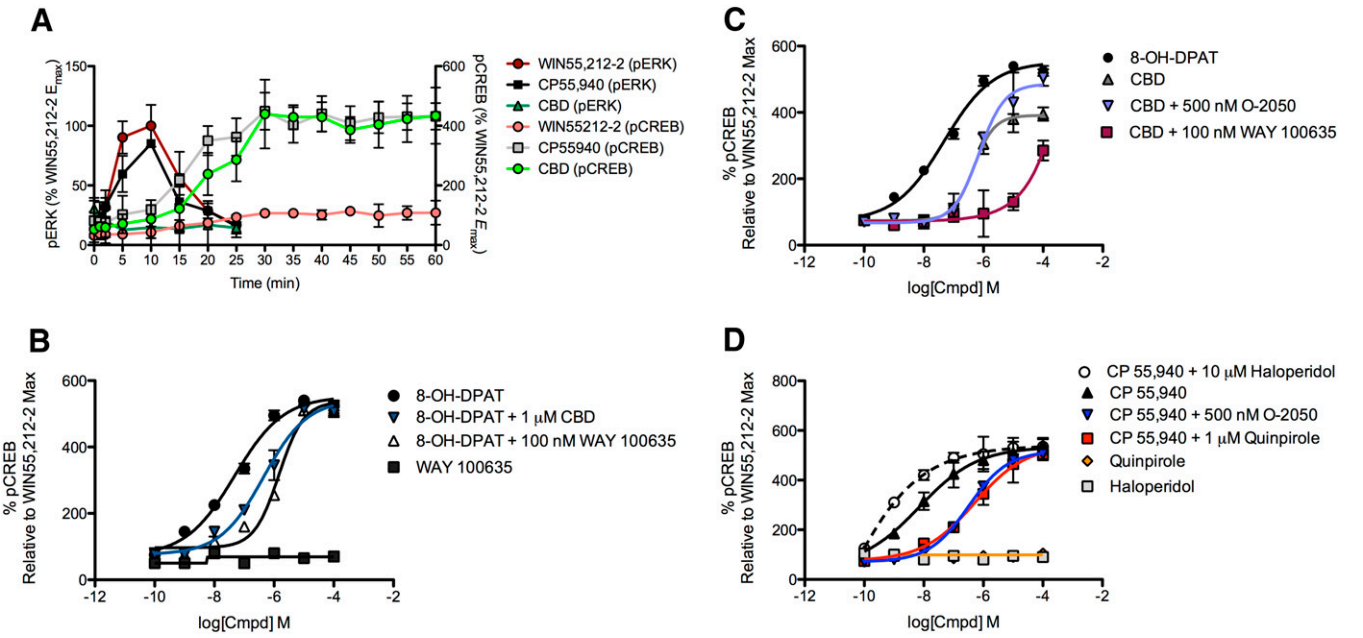

Fig. 5. CB_1-independent CREB signaling. (A) Time course of ERK and CREB signaling. ST*HdhQ7/Q7* cells were treated with 1 μM WIN55,212-2, CP55940, or CBD for 0–60 min, and ERK (left y-axis) or CREB (right y-axis) phosphorylation was measured via In-Cell Western. $N = 4$. (B,C) $5HT_{1A}$-dependent CREB signaling. ST$Hdh^{Q7/Q7}$ cells were treated with 0.1–100,000 nM 8-OH-DPAT, WAY-100,635, or CBD, ± 1 μM CBD, 100 nM WAY-100,635, or 500 nM O-2050 for 30 minutes, and CREB phosphorylation was measured via In-Cell Western. $N = 4$. (D) D_2-dependent CREB signaling ST$Hdh^{Q7/Q7}$ cells were treated with 0.1–100,000 nM CP, quinpirole, or haloperidol, ± 10 μM haloperidol, 1 μM quinpirole, or 500 nM O-2050 for 30 minutes and CREB phosphorylation was measured via In-Cell Western. Concentration-response curves were fit using non-linear regression models. $N = 4$. All data are expressed relative to WIN E_{max} in ST$Hdh^{Q7/Q7}$ cells.

data provide support for $G\alpha_{i/o}$- and $G\beta\gamma$-selective activation of CB_1 to maintain CB_1 levels and the cellular function and viability of cells expressing mHtt (Blázquez et al., 2011, 2015; Mievis et al., 2011; Horne et al., 2013; Chiarlone et al., 2014; Naydenov et al., 2014).

Use of THC and CBD in HD. Despite a lack of clinical evidence, patients suffering from HD may be seeking medical marijuana or acquiring it from other sources in an attempt to relieve some of the symptoms of their disease (Müller-Vahl et al., 1999; Meisel and Friedman, 2012; Koppel et al., 2014). Most medically available and tested illicit marijuana contains a high concentration of THC relative to other cannabinoids, such as CBD (De Backer et al., 2012). Here, we observed that THC reduced cellular function and viability in cells expressing mHtt whether THC was used alone or in a 1:1 combination with CBD. Likewise, treatment of R6/1 and R6/2 mouse models with 10 mg/kg THC is associated with worsening of HD signs and symptoms (Dowie et al., 2010). However, others have reported improvement in motor control and reduced striatal atrophy in R6/1 and R6/2 HD treated for 6 weeks with 2 mg/kg THC beginning at 4 weeks of age (Blázquez et al., 2011), suggesting that the deleterious effects of THC in HD are dose- and time course–dependent. CBD alone displayed mixed beneficial and negative effects in ST$Hdh^{Q7/Q7}$ and ST$Hdh^{Q111/Q111}$ cells. CBD is known to act through a number of effectors, including as a negative allosteric modulator at CB_1 and a partial agonist at $5HT_{1A}$ (Pazos et al., 2013; Laprairie et al., 2015). It is unclear which effects of CBD predominate in vivo normally and in HD and how the combinations of any or all of the at least 65 cannabinoids found in marijuana (McPartland et al., 2015) influence one another's pharmacokinetics and pharmacodynamics (Sagredo et al., 2011; Valdeolivas et al., 2012). Further, the utility of CBD in HD remains controversial, with some studies reporting no effects in animal models and human trials (Consroe et al., 1991; Valdeolivas et al., 2012), or positive effects in animal models (Sagredo et al., 2007, 2011). Overall, the use of THC or marijuana may exacerbate the signs and symptoms of HD via further downregulation of CB_1 and reduced cellular viability.

Conclusions

$G\alpha_{i/o}$- and $G\beta\gamma$-selective CB_1 ligands are probably the most therapeutically useful cannabinoids in the treatment of HD. However, highly potent synthetic cannabinoids, such as WIN, may produce unwanted psychoactive effects and their chronic use would probably result in receptor desensitization or downregulation (Sim-Selley and Martin, 2002; Blair et al., 2009). Endocannabinoids, which we observed to enhance $G\alpha_{i/o}$- and $G\beta\gamma$-dependent signaling in the ST*Hdh* cell culture system, are rapidly metabolized in vivo and consequently have limited efficacy when they are directly administered (Devane et al., 1992; Kondo et al., 1998). The inhibitor of endocannabinoid catabolism URB597 has demonstrated limited efficacy at improving motor control deficits in R6/2 HD mice (Dowie et al., 2010), but additional studies are needed to understand how elevating endocannabinoid levels affects the signs and symptoms of HD in vivo. An alternative means of enhancing endogenous CB_1 signaling is with the use of positive allosteric modulators (PAMs) of CB_1. PAMs bind to a site on the receptor that is distinct from the site of endogenous ligand binding (i.e., the orthosteric site) and enhance the binding and efficacy of the endogenous ligands that are produced and regulated through intrinsic control mechanisms (Pamplona et al.,

2012; Wootten et al., 2013). CB_1 PAMs may increase $G\alpha_{i/o}$-dependent pro-survival signaling occurring via endocannabinoids without producing the psychotropic effects associated with synthetic cannabinoid agonists, because they are unable to directly activate CB_1. Our in vitro study of cannabinoid functional selectivity leads us to conclude that enhancement of endocannabinoid-dependent CB_1 activation may be a means of treating the signs and symptoms of HD by targeting CB_1.

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Authorship Contributions

Participated in research design: Laprairie, Bagher, Kelly, and Denovan-Wright.

Conducted experiments: Laprairie.

Contributed new reagents or analytic tools: Kelly, Denovan-Wright.

Performed data analysis: Laprairie, Denovan-Wright.

Wrote or contributed to the writing of the manuscript: Laprairie, Bagher, Kelly, Denovan-Wright.

References

Bagher AM, Laprairie RB, Kelly ME, and Denovan-Wright EM (2013) Co-expression of the human cannabinoid receptor coding region splice variants (hCB_1) affects the function of hCB_1 receptor complexes. *Eur J Pharmacol* **721**:341–354.

Benn CL, Slow EJ, Farrell LA, Graham R, Deng Y, Hayden MR, and Cha JH (2007) Glutamate receptor abnormalities in the YAC128 transgenic mouse model of Huntington's disease. *Neuroscience* **147**:354–372.

Black JW and Leff P (1983) Operational models of pharmacological agonism. *Proc R Soc Lond B Biol Sci* **220**:141–162.

Blair RE, Deshpande LS, Sombati S, Elphick MR, Martin BR, and DeLorenzo RJ (2009) Prolonged exposure to WIN55,212–2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. *Neuropharmacology* **57**: 208–218.

Blázquez C, Chiarlone A, Sagredo O, Aguado T, Pazos MR, Resel E, Palazuelos J, Julien B, Salazar M, and Börner C et al. (2011) Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. *Brain* **134**:119–136.

Blázquez C, Chiarlone A, Bellocchio L, Resel E, Pruunsild P, García-Rincón D, Sendtner M, Timmusk T, Lutz B, and Galve-Roperh I et al. (2015) The CB_1 cannabinoid receptor signals striatal neuroprotection via a PI3K/Akt/mTORC1/BDNF pathway. *Cell Death Differ* **22**:1618–1629.

Botelho EP, Wang E, Chen JY, Holley S, Andre V, Cepeda C, and Levine MS (2014) Differential synaptic and extrasynaptic glutamate-receptor alterations in striatal medium-sized spiny neurons of aged yac128 Huntington's disease Mice. *PLoS Curr* 6 DOI: 10.1371/currents.hd.34957c4f8bd7cb1f5ec47381dfc811c3.

Chiarlone A, Bellocchio L, Blázquez C, Resel E, Soria-Gómez E, Cannich A, Ferrero JJ, Sagredo O, Benito C, and Romero J et al. (2014) A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proc Natl Acad Sci USA* **111**: 8257–8262.

Christopoulos A (2014) Advances in G protein-coupled receptor allostery: from function to structure. *Mol Pharmacol* **86**:463–478.

Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R, Kennedy K, and Schram K (1991) Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacol Biochem Behav* **40**:701–708.

De Backer B, Maebe K, Verstraete AG, and Charlier C (2012) Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of plants. *J Forensic Sci* **57**:918–922.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**:1946–1949.

Denovan-Wright EM and Robertson HA (2000) Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience* **98**:705–713.

Dowie MJ, Howard ML, Nicholson LF, Faull RL, Hannan AJ, and Glass M (2010) Behavioural and molecular consequences of chronic cannabinoid treatment in Huntington's disease transgenic mice. *Neuroscience* **170**:324–336.

Ehlert FJ (2015) Functional studies cast light on receptor states. *Trends Pharmacol Sci* **36**:596–604.

Ehlert FJ, Suga H, and Griffin MT (2011) Quantifying agonist activity at G protein-coupled receptors. *J Vis Exp* **58**:e3179.

Fernández-Ruiz J (2009) The endocannabinoid system as a target for the treatment of motor dysfunction. *Br J Pharmacol* **156**:1029–1040.

Glass M and Felder CC (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci* **17**:5327–5333.

Glass M, Dragunow M, and Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* **97**:505–519.

Griffin MT, Figueroa KW, Liller S, and Ehlert FJ (2007) Estimation of agonist activity at G protein-coupled receptors: analysis of M2 muscarinic receptor signaling through Gi/o,Gs, and G15. *J Pharmacol Exp Ther* **321**:1193–1207.

Hayakawa K, Mishima K, Irie K, Hazekawa M, Mishima S, Fujioka M, Orito K, Egashira N, Katsurabayashi S, and Takasaki K et al. (2008) Cannabidiol prevents a post-ischemic injury progressively induced by cerebral ischemia via a high-mobility group box1-inhibiting mechanism. *Neuropharmacology* **55**:1280–1286.

Horne EA, Coy J, Swinney K, Fung S, Cherry AE, Marrs WR, Naydenov AV, Lin YH, Sun X, and Keene CD et al. (2013) Downregulation of cannabinoid receptor 1 from neuropeptide Y interneurons in the basal ganglia of patients with Huntington's disease and mouse models. *Eur J Neurosci* **37**:429–440.

James JR, Oliveira MI, Carmo AM, Iaboni A, and Davis SJ (2006) A rigorous experimental framework for detecting protein oligomerization using bioluminescence resonance energy transfer. *Nat Methods* **3**:1001–1006.

Kathmann M, Flau K, Redmer A, Tränkle C, and Schlicker E (2006) Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* **372**:354–361.

Kearn CS, Blake-Palmer K, Daniel E, Mackie K, and Glass M (2005) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* **67**:1697–1704.

Kenakin T and Christopoulos A (2013) Measurements of ligand bias and functional affinity. *Nat Rev Drug Discov* **12**:483.

Kenakin T, Watson C, Muniz-Medina V, Christopoulos A, and Novick S (2012) A simple method for quantifying functional selectivity and agonist bias. *ACS Chem Neurosci* **3**: 193–203.

Khajehali E, Malone DT, Glass M, Sexton PM, Christopoulos A, and Leach K (2015) Biased agonism and biased allosteric modulation at the CB1 cannabinoid receptor. *Mol Pharmacol* **88**:368–379.

Kloster E, Saft C, Epplen JT, and Arning L (2013) CNR1 variation is associated with the age at onset in Huntington disease. *Eur J Med Genet* **56**:416–419.

Kondo S, Kondo H, Nakane S, Kodaka T, Tokumura A, Waku K, and Sugiura T (1998) 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through CA2+-dependent and -independent mechanisms. *FEBS Lett* **429**:152–156.

Koppel BS, Brust JC, Fife T, Bronstein J, Youssof S, Gronseth G, and Gloss D (2014) Systematic review: efficacy and safety of medical marijuana in selected neurologic disorders: report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology* **82**:1556–1563.

Kumar A, Kumar Singh S, Kumar V, Kumar D, Agarwal S, and Rana MK (2015) Huntington's disease: an update of therapeutic strategies. *Gene* **556**:91–97.

Laprairie RB, Kelly ME, and Denovan-Wright EM (2013) Cannabinoids increase type 1 cannabinoid receptor expression in a cell culture model of striatal neurons: implications for Huntington's disease. *Neuropharmacology* **72**:47–57.

Laprairie RB, Bagher AM, Kelly ME, Dupré DJ, and Denovan-Wright EM (2014) Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. *J Biol Chem* **289**:24845–24862.

Laprairie RB, Bagher AM, Kelly ME, and Denovan-Wright EM (2015) Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br J Pharmacol* **172**:4790–4805.

Lauckner JE, Hille B, and Mackie K (2005) The cannabinoid agonist WIN55,212–2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc Natl Acad Sci USA* **102**:19144–19149.

Luttrell LM, Maudsley S, and Bohn LM (2015) Fulfilling the promise of "biased" G protein-coupled receptor agonism. *Mol Pharmacol* **88**:579–588.

MacCoubrey IC, Moore PL, and Haugland RP (1990) Quantitative fluorescence measurements of cell viability (cytotoxicity) with a multi-well plate scanner. *J Cell Biol* **111**:303.

McCaw EA, Hu H, Gomez GT, Hebb AL, Kelly ME, and Denovan-Wright EM (2004) Structure, expression and regulation of the cannabinoid receptor gene (CB1) in Huntington's disease transgenic mice. *Eur J Biochem* **271**:4909–4920.

McKenzie FR and Milligan G (1991) Cholera toxin impairment of opioid-mediated inhibition of adenylate cyclase in neuroblastoma x glioma hybrid cells is due to a toxin-induced decrease in opioid receptor levels. *Biochem J* **275**:175–181.

McPartland JM, Duncan M, Di Marzo V, and Pertwee RG (2015) Are cannabidiol and Δ(9) -tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol* **172**:737–753.

Meisel K and Friedman JH (2012) Medical marijuana in Huntington's disease: report of two cases. *Med Health R I* **95**:178–179.

Mievis S, Blum D, and Ledent C (2011) Worsening of Huntington disease phenotype in CB1 receptor knockout mice. *Neurobiol Dis* **42**:524–529.

Milligan G, Unson CG, and Wakelam MJ (1989) Cholera toxin treatment produces down-regulation of the alpha-subunit of the stimulatory guanine-nucleotide-binding protein (Gs). *Biochem J* **262**:643–649.

Müller-Vahl KR, Kolbe H, Schneider U, and Emrich HM (1999) Cannabis in movement disorders. *Forsch Komplementarmed* **6** (Suppl 3):23–27.

Naydenov AV, Sepers MD, Swinney K, Raymond LA, Palmiter RD, and Stella N (2014) Genetic rescue of CB1 receptors on medium spiny neurons prevents loss of excitatory striatal synapses but not motor impairment in HD mice. *Neurobiol Dis* **71**:140–150.

Pamplona FA, Ferreira J, Menezes de Lima O, Jr, Duarte FS, Bento AF, Forner S, Villarinho JG, Bellocchio L, Wotjak CT, and Lerner R et al. (2012) Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proc Natl Acad Sci USA* **109**:21134–21139.

Paoletti P, Vila I, Rifé M, Lizcano JM, Alberch J, and Ginés S (2008) Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: the role of p25/cyclin-dependent kinase 5. *J Neurosci* **28**:10090–10101.

Pazos MR, Mohammed N, Lafuente H, Santos M, Martínez-Pinilla E, Moreno E, Valdizan E, Romero J, Pazos A, and Franco R et al. (2013) Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. *Neuropharmacology* **71**:282–291.

Pertwee RG (2008) Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol* **13**:147–159.
Russo EB, Burnett A, Hall B, and Parker KK (2005) Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* **30**:1037–1043.
Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, and Greasley PJ (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**:1092–1101.
Sadri-Vakili G, Menon AS, Farrell LA, Keller-McGandy CE, Cantuti-Castelvetri I, Standaert DG, Augood SJ, Yohrling GJ, and Cha JH (2006) Huntingtin inclusions do not down-regulate specific genes in the R6/2 Huntington's disease mouse. *Eur J Neurosci* **23**:3171–3175.
Sagredo O, Pazos MR, Satta V, Ramos JA, Pertwee RG, and Fernández-Ruiz J (2011) Neuroprotective effects of phytocannabinoid-based medicines in experimental models of Huntington's disease. *J Neurosci Res* **89**:1509–1518.
Sagredo O, Ramos JA, Decio A, Mechoulam R, and Fernández-Ruiz J (2007) Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *Eur J Neurosci* **26**:843–851.
Sim-Selley LJ and Martin BR (2002) Effect of chronic administration of R-(+)-[2,3-Dihydro–5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55,212–2) or delta(9)-tetrahydrocannabinol on cannabinoid receptor adaptation in mice. *J Pharmacol Exp Ther* **303**:36–44.
Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, Cattaneo E, and MacDonald ME (2000) Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. *Hum Mol Genet* **9**:2799–2809.
Valdeolivas S, Satta V, Pertwee RG, Fernández-Ruiz J, and Sagredo O (2012) Sativex-like combination of phytocannabinoids is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease: role of CB1 and CB2 receptors. *ACS Chem Neurosci* **3**:400–406.
Van Laere K, Casteels C, Dhollander I, Goffin K, Grachev I, Bormans G, and Vandenberghe W (2010) Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. *J Nucl Med* **51**:1413–1417.
Wootten D, Christopoulos A, and Sexton PM (2013) Emerging paradigms in GPCR allostery: implications for drug discovery. *Nat Rev Drug Discov* **12**:630–644.

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