Autoradiography Methodologies in CNS Research

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1 INTRODUCTION

Autoradiography is a powerful technique that can be applied to study cerebral blood flow, receptor density and activation of G protein-coupled receptors (GPCRs) by novel pharmacological compounds in the brain. These methodologies are easily applicable to various disease models, and combining them with behavioural readouts allows a versatile evaluation of pathophysiology of the CNS disorders and mechanisms of action of novel therapies.

GPCRs are involved in a wide variety of physiological processes, including regulation of behaviour and mood. They activate intercellular signal transduction pathways and cellular responses. Cannabinoid, serotonin, dopamine, $GABA_B$, and metabotropic glutamate receptors are among neurologically interesting GPCR targets. Ligand binding to GPCRs induces an interaction of the receptor with G protein that stimulates the release of GDP simultaneously with the exchange of GTP. ^{35}S-GTPγS autoradiography has been applied to study receptor activation after ligand binding to GPCRs of Gi and Gs types.

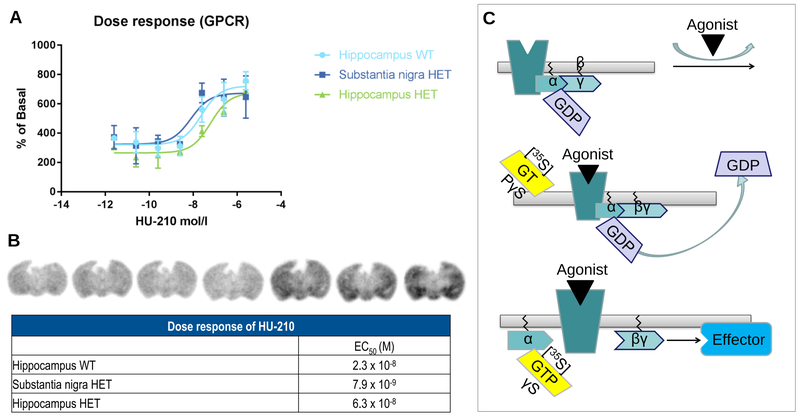
In this study, we analyzed changes in the receptor density in rodent neurological disease models. To assess receptor density alterations, tritiated ligands to dopamine receptors 1 and 2, serotonin transporters and cannabinoid receptor 1 (CB1) were used and Bmax values of corresponding ligands were determined in relevant brain regions. Changes in Bmax values correlate with alterations in the number of available receptors sites and which may correlate with the phase or severity of the disease.

We also determined the dose-response relationship of GPCR activation by the CB1 agonist HU-210 by detecting consumption of GTPγ^{35}S in the hippocampus and substantia nigra in mice. The potency (half maximal effective concentration, EC50) of interaction of HU-210 with CB1 was calculated from the obtained dose-response curves.

The current examples demonstrate how a combination of assays can be utilized to advance our understanding of the disease state and measure effects of pharmacological treatments. Also, applied digital scintillation autoradiography enables quantitative and real-time imaging of tritiated samples within hours, compared to minimum of several weeks of exposure time required to phosphoscreens or films. This accelerates method development and analysis substantially. As a summary, the combination of receptor and functional autoradiography offers a powerful tool to comprehensively measure changes in disease models or responses to novel molecules.

2 GPCR ACTIVATION

Dose-response relationship of GPCR activation in hippocampus and substantia nigra was studied in Q175 mice at the age of 9 mo (Figure 1). Briefly, sections were incubated using various concentrations of CB1 agonist, HU-210. To confirm receptor binding site, Rimonabant was used as antagonist.

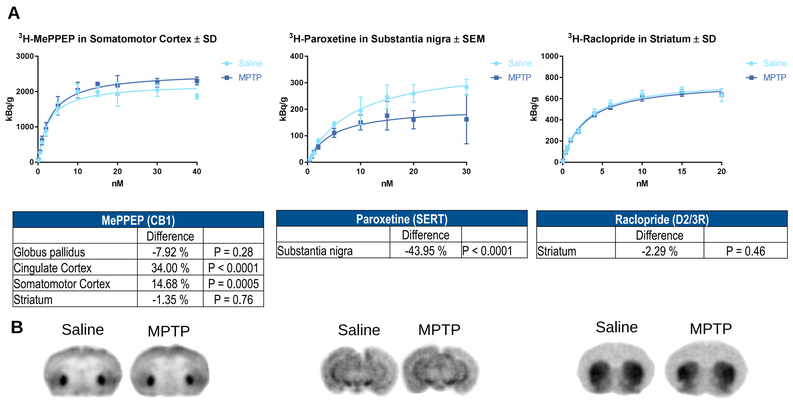


Dose response of HU-210	
	EC_{50} (M)
Hippocampus WT	2.3 x 10^{-8}
Substantia nigra HET	7.9 x 10^{-9}
Hippocampus HET	6.3 x 10^{-8}

Figure 1. A) To study potency of HU-210 in hippocampus and substantia nigra, mouse brain sections were incubated with increasing agonist concentration. Antagonist (rimonabant) was used as 10^{-6} M for each sample. B) Example images of HU-210 activation with increasing concentration (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) and EC50 values for studied regions. C) Binding of a non-hydrolyzable GTP analogue to a receptor containing GPCR. Binding of receptor agonist activates GPCR, enabling nucleotide exchange. Hydrolysis of ^{35}S-GTPγS does not occur as normal GTP due to replacement of oxygen by sulphur in third phosphate group, which can be utilized as functional autoradiography.

3 PARKINSON'S DISEASE

Loss of dopaminergic cells in Parkinson's disease was modelled with MPTP challenge. After in vivo part of the experiment, the brains were collected and maximal binding (Bmax) of tritiated radioligands to corresponding receptors in cortex, striatum, globus pallidus and substantia nigra was determined using autoradiography (Figure 2).



MePPEP (CB1)		
	Difference	
Globus pallidus	-7.92 %	P = 0.28
Cingulate Cortex	34.00 %	P < 0.0001
Somatomotor Cortex	14.68 %	P = 0.0005
Striatum	-1.35 %	P = 0.76

Paroxetine (SERT)		
	Difference	
Substantia nigra	-43.95 %	P < 0.0001

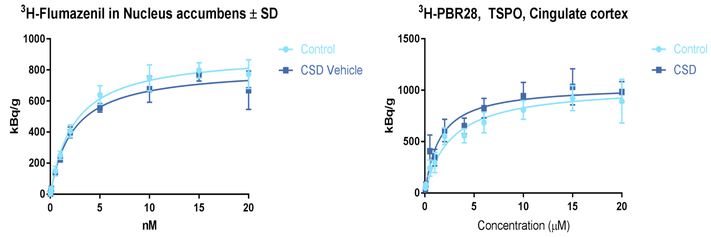
Raclopride (D2/3R)		
	Difference	
Striatum	-2.29 %	P = 0.46

Figure 2. A) Various receptor densities between MPTP (n=4) and vehicle (n=4) treated mice were studied using Bmax assay of radioligand to its target receptor and autoradiography. As a summary, significant (p<0.05) decrease of SERT (SNC), increase of CB1 (Cing & SomCortex) and increase of GABA (CingCortex) in MPTP mice was observed compared to naïve. No difference in D2/3R receptor density between MPTP and naive mice was observed. B) Representative ARG images of saline and MPTP treated mice from CB1 (globus pallidus), SERT (substantia nigra) and D2/3R (striatum).

4 DEPRESSION

The chronic social defeat (CSD) model is ethologically relevant and has robust depressive-like endpoints. Numerous brain areas show altered activity in patients suffering from depression, and this has encouraged advocates of various theories that seek to identify a biochemical origin of the disease, as opposed to theories that emphasize psychological or situational causes.

Several neuromodulators are altered in depressive patients. For that reason we looked the possible alterations in several receptor levels in CSD model (Figure 3). As a summary, $GABA_A$ was decreased in nucleus accumbensis, CB1 was increased in cingulate cortex, SERT was increased in substantia nigra in CSD mice compared to naïve animals.



Flumazenil (GABA)		
	Difference	
Cingulate Cortex	1.92 %	P = 0.53
Somatomotor Cortex	0.14 %	P = 0.96
Striatum	-5.30 %	P = 0.15
Nucleus accumbens	-10.17 %	P = 0.01

MePPEP (CB1)		
	Difference	
Globus pallidus	0.37 %	P = 0.96
Cingulate Cortex	13.69 %	P = 0.001
Somatomotor Cortex	4.29 %	P = 0.22
Striatum	3.00 %	P = 0.45

Paroxetine (SERT)		
	Difference	
Substantia nigra	-28.96 %	P = 0.11
Amygdala	-16.75 %	P = 0.15

PBR28 (TSPO)		
	Difference	
Cingulate cortex	+8.3%	P = 0.35
Insular cortex	-0.3%	P=0.98

Raclopride (D2/3R)		
	Difference	
Striatum	1.48 %	P = 0.48

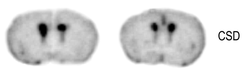


Figure 3. Various receptor densities between CSD (n=4) and naive (n=4) mice were studied using Bmax assay of radioligand to its target receptor and autoradiography. As a summary, significant (p<0.05) decrease of GABA (NuclAcc) and increase of CB1 (CingCortex) in CSD mice was observed compared to naïve. Representative ARG images of saline and MPTP treated mice with TSPO radioligand (globus pallidus).

5 HUNTINGTON'S DISEASE

Huntington's disease (HD) is a neurodegenerative genetic disorder that affects the brain, muscle coordination and leads to cognitive decline and psychiatric problems. In HD patients, several changes in neurotransmitter receptors, like glutamate, dopamine, GABA, muscarinic, cholinergic and adenosine receptors have been reported. In the current studies, we compared receptor densities of and gamma-aminobutyric acid ($GABA_A$) cannabinoid 1 (CB1) in Huntington's disease (HD) mouse model using wild type (WT) and Q175 KI heterozygote (HET) mice.

Age	Region	Change in Bmax	P-value (F-test)
3 months	Globus pallidus	-6.2%	P = 0.0117
	Substantia nigra	11.8%	P = 0.0041
	Striatum	9.3%	P < 0.0001
	Somatomotor cortex	-0.1%	P = 0.6763
	Cingulate cortex	-2.0%	P = 0.5437
6 months	Globus pallidus	-7.9%	P = 0.0165
	Substantia nigra	-7.4%	P = 0.0543
	Striatum	28.6%	P < 0.0001
	Somatomotor cortex	-0.1%	P = 0.9599
	Cingulate cortex	-2.0%	P = 0.3800
9 months	Globus pallidus	20.7%	P = 0.0001
	Substantia nigra	15.6%	P < 0.0001
	Striatum	35.5%	P < 0.0001
	Somatomotor cortex	7.6%	P = 0.0668
	Cingulate cortex	8.9%	P = 0.0181
12 months	Globus pallidus	0.4%	P = 0.8597
	Substantia nigra	-2.7%	P = 0.4651
	Striatum	33.1%	P < 0.0001
	Somatomotor cortex	-1.0%	P = 0.6711
	Cingulate cortex	6.0%	P = 0.0545
15 months	Globus pallidus	13.5%	P < 0.0001
	Substantia nigra	3.8%	P = 0.3827
	Striatum	41.0%	P < 0.0001
	Somatomotor cortex	5.6%	P = 0.1169
	Cingulate cortex	4.3%	P = 0.1992

Table 1. Summarized GABA analysis from studied brain regions of interest using ^{3}H-flumazenil in zQ175KI WT and HET male mice (n=24/age group; 12/genotype). P-value from extra sum-of-squares F-test indicates whether Bmax parameters differ (p<0.05) between genotypes.

	Region	Change in Bmax	P-value (F-test)
2 months	Globus pallidus	0.9%	P = 0.8664
	Substantia nigra	9.4%	P = 0.0741
	Striatum	-2.1%	P = 0.5679
	Somatomotor cortex	-4.0%	P = 0.1395
	Cingulate cortex	-7.1%	P = 0.0096
4 months	Globus pallidus	-33.4%	P < 0.0001
	Substantia nigra	-2.9%	P = 0.5334
	Striatum	-6.2%	P = 0.0514
	Somatomotor cortex	-11.1%	P = 0.0004
	Cingulate cortex	-6.4%	P = 0.0624
6 months	Globus pallidus	-41.8%	P < 0.0001
	Substantia nigra	-44.2%	P < 0.0001
	Striatum	-7.7%	P = 0.0862
	Somatomotor cortex	-1.7%	P = 0.6531
	Cingulate cortex	-7.9%	P = 0.0458
9 months	Globus pallidus	-38.8%	P < 0.0001
	Substantia nigra	-35.7%	P < 0.0001
	Striatum	-15.3%	P = 0.0010
	Somatomotor cortex	-1.6%	P = 0.7510
	Cingulate cortex	-1.4%	P = 0.6832
12 months	Globus pallidus	-47.1%	P < 0.0001
	Substantia nigra	-60.1%	P < 0.0001
	Striatum	-17.5%	P < 0.0001
	Somatomotor cortex	-5.1%	P = 0.0615
	Cingulate cortex	-9.6%	P = 0.0004
15 months	Globus pallidus	-57.2%	P < 0.0001
	Substantia nigra	-50.2%	P < 0.0001
	Striatum	-12.2%	P = 0.0131
	Somatomotor cortex	-5.7%	P = 0.2198
	Cingulate cortex	-1.0%	P = 0.8219

Table 2. Summarized CB1 analysis from studied brain regions of interest using ^{3}H-flumazenil in zQ175KI WT and HET male mice (n=24/age group; 12/genotype). P-value from extra sum-of-squares F-test indicates whether Bmax parameters differ (p<0.05) between genotypes.

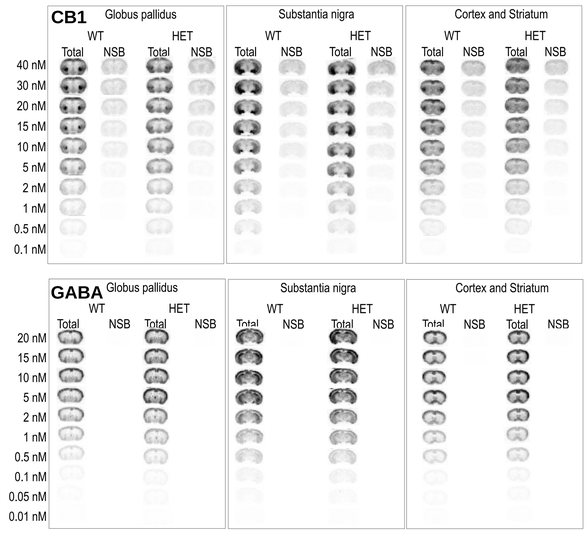


Figure 4. Example autoradiographs from WT and HET zQ175 KI mice with increasing concentrations of radioligands to CB1 (^{3}H-MePPEP) or GABA (^{3}H-Flumazenil) at 15 mo of age. Nonspecific binding was minimal with all used concentrations.

6 CONCLUSION

The current data demonstrate how the combination of assays can be utilized to advance our understanding of disease state and effect of treatment. Also, measuring both receptor density and downstream signaling pathway are vital in understanding both the pathophysiology of the disease and drug mechanism of action. As a summary, the combination of receptor and functional autoradiography offers a powerful tool to comprehensively measure changes in disease models or responses to novel molecules.

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