Occupy of Nociceptin/Orphanin FQ Peptide Receptors by the Antagonist LY2940094 in Rats and Healthy Human Subjects

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ABSTRACT

Therapeutic benefits from nociceptin opioid peptide receptor (NOP) antagonism were proposed for obesity, eating disorders, and depression. LY2940094 [(2-[4-[(2-chloro-4,4-difluoro-spiro[5H-thieno[2,3-c]pyran-7,4-piperidine]-1-yl)methyl]-3-methyl-4-pyrazol-1-yl]-3-pyridyl]methanol) is a novel, orally bioavailable, potent, and selective NOP antagonist. We studied NOP receptor occupancy (RO) after single oral LY2940094 doses in rat hypothalamus and human brain by use of liquid chromatography with tandem mass spectrometry (LC-MS/MS) (LSN2810397) and positron emission tomography (PET) (11C)NOP-1A) tracers, respectively. A bolus plus constant infusion tracer protocol with PET was employed in humans at 2.5 and 26.5 hours after administration of the LY2940094 dose. The RO was calculated from the change in regional distribution volume (VR) corrected for nondisplaceable volume using Lasson plots. The RO followed a simple Emax relationship to plasma LY2940094 concentration, reaching near complete occupancy in both species. For rat hypothalamus, the plasma concentration at half-maximum RO (EC50) was 5.8 ng/ml. In humans, LY2940094 was well tolerated and safe over the 4–40 mg dose range, and it peaked in plasma at 2 to 6 hours after a 1- to 2-hour lag, with approximate dose-proportional exposure. After 4–40 mg doses, NOP RO was similar across the prefrontal cortex, occipital cortex, putamen, and thalamus, with EC50 of 2.94 to 3.46 ng/ml, less than 2-fold lower than in rats. Over 4–40 mg doses, LY2940094 mean plasma levels at peak and 24 hours were 7.93–102 and 1.17–14 ng/ml, corresponding to the cross-region average NOP RO of 73–97% and 28–82%, respectively. The rat EC50 translates well to humans. LY2940094 readily penetrates the human brain, and a once-daily oral dose of 40 mg achieves sustainably high (>80%) NOP RO levels suitable for testing clinical efficacy.

Introduction

Nociceptin/orphanin FQ (nociceptin) is a 17-amino acid neuropeptide that acts as an endogenous ligand for the opioid-like one receptor (ORL1), also known as NOP (nociceptin opioid peptide receptor). NOP is a class A G-protein–coupled receptor (Meunier et al., 1995; Reinscheid et al., 1995), is encoded by the gene opiate receptor-like 1 (OPRL1), and is widely expressed in the central nervous system and specifically in regions associated with mood disorders and obesity. Symptoms of many neuropsychiatric disorders, such as pain, anxiety, depression, anorexia, obesity, and drug abuse, are believed to be potentially linked to the NOP receptor (Lambert, 2008; Murphy, 2010), which suggests NOP antagonism as a potential therapeutic strategy (Gavioli and Calo, 2006; Witkin et al., 2014).

Interest in the target has spurred the discovery of multiple tracers. Hostetler et al. (2013) reported the discovery of [11F]MK-0911, a potent, selective, and brain-penetrant positron emission tomography (PET) tracer, which was shown to have NOP binding in monkeys and humans, and was displacable by the potent NOP antagonist MK-5757. We have recently discovered both labeled and unlabeled NOP receptor tracers with subnanomolar binding affinities with no intrinsic activity (i.e., antagonists), high selectivity, central nervous system penetration, and low nonspecific binding (Pedregal et al., 2012). LSN2810397 [Compound (S)-27 in Pedregal et al., 2012; NOP Ki = 0.114 nM] is an unlabeled tracer based on liquid chromatography with tandem mass spectrometry (LC-MS/MS), and [13C]NOP-1A (Kimura et al., 2011; Lohith et al., 2012, 2014; Pike et al., 2011) is a labeled tracer (NOP Ki = 0.15 nM) that lacks brain-penetrant radiomatabilites and is suitable for

ABBREVIATIONS: AE, adverse event; CAMH, Centre for Addiction and Mental Health; Cₚ, tracer concentration in plasma; Cᵣ, average tracer concentration in the brain; EC₅₀, plasma concentration at half-maximum receptor occupancy; Eᵢₘₐₓ, maximum occupancy; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LY2940094, [2-4-[(2-chloro-4,4-difluoro-spiro[5H-thieno[2,3-c]pyran-7,4-piperidine]-1-yl)methyl]-3-methyl-4-pyrazol-1-yl]-3-pyridyl]methanol; MRI, magnetic resonance imaging; NAD-299, (3R)-3-[dicyclobutylamino]-8-fluoro-3,4-dihydro-2H-chromene-5-carboxamide; NOP, nociceptin opioid peptide receptor; PET, positron emission tomography; RO, receptor occupancy; Ro-64-6198, [(1S,3aS)-2,3a,3,4,5,6-hexahydro-1H-phenalen-1-yl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one; ROI, regions of interest; SB-612111, (S,S,S)-7-[(4-2,6-dichlorophenyl)piperidin-1-yl][methyl]-1-methyl-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol; TEAE, treatment-emergent adverse event; VR, PET tracer distribution volume.
PET studies. The two tracers are similar structurally, differing only in the presence or absence of one N-substituted methyl. In humans, [11C]NOP-1A is safe, with favorable kinetics and reproducibility (Lohith et al., 2012, 2014), and exhibits a distribution volume (V2) consistent with known NOP density distribution (Berthole et al., 2003).

Potent and selective NOP antagonists based on the dihydrospiro (piperidine-4,7’-thieno[2,3-c]pyran) chemical scaffold have been recently discovered (Toledo et al., 2014). Among these, {2-[4-(2,6-dichlorophenyl)-4,4-difluoro-spiro[5F-thieno[2,3-c]pyran-7,4’-piperidine]-1’-yl]-methyl}-3-methyl-pyrazol-1-yl]-3-pyridyl)methanol, referred to as LY2940094 (Statnick et al., 2016) (Compound 36 in Toledo et al., 2014; NOP Ki = 0.105 nM), was advanced to clinical development for the treatment of depression and alcohol dependence.

We assessed the brain NOP receptor occupancy (RO) after single oral administration of a range of doses of LY2940094 to healthy volunteers using [11C]NOP-1A as a PET ligand, and examined the relationship between the NOP RO levels of LY2940094 and its plasma concentrations to support dose selection for future efficacy trials. Furthermore, we assessed the translatability of the concentration-RO relationship of LY2940094 between that in rats (using LSN2810397 as the LC-MS/MS-based tracer) and humans (using [11C]NOP-1A and PET imaging).

Materials and Methods

Preclinical Studies of LY2940094 NOP RO in Rats. The NOP RO of LY2940094 (Eli Lilly and Company, Indianapolis, IN) was evaluated in the hypothalamus in both dose-response and time-course experiments. Male Sprague-Dawley rats (230–300 g; three to four per dose group or time point) were housed on a 12-hour light/dark cycle (testing during light phase) and received free access to normal rat chow and water. Animals received either vehicle, the reference NOP antagonist (53.75-7-[4-(2,6-dichlorophenyl)-piperidin-1-yl]-methyl]-1-methyl-6,7,8,9-tetrahydro-SH-benzo[7]annulen-5-ol (SB-612111, 30 mg/kg, oral by gavage; Eli Lilly and Company) as a positive control (Spagnolo et al., 2007), or LY2940094 (0.01–10 mg/kg for the dose-response experiment; 1 mg/kg for the time-course experiment). For the dose–response experiment, NOP RO was assessed at 6 hours after dose administration; for the time-course experiment, NOP RO was assessed at 0.083, 0.25, 1, 2, 4, 8, and 24 hours after dose administration. All experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals under protocols approved by a local animal care and use committee.

For every RO assessment, 3 μg/kg of the tracer LSN2810397 (Eli Lilly and Company) was administered intravenously (Eli Lilly and Company, Indianapolis, IN) in the antecubital vein, during which the radiotracer is known to be stable. The average total injected dose of radioactivity before and after each scan as well as serially over a 24-hour period. LY2940094 injection can be found in Supplemental Fig. 3. The emission PET images were acquired on a whole body PET camera system, Siemens Biograph HiRez XVI (Siemens Molecular Imaging, Knoxville, TN). Magnetic resonance imaging (MRI) scans were obtained before the PET scanning procedures to coregister the PET and MRI images for analysis of the PET data to identify the volumes of interest using an automated method verified by visual inspection (Rusjan et al., 2006). Additional details regarding the MRI and PET image acquisition can be found in Supplemental Fig. 3.

Distribution Volume and RO Determination. PET tracer distribution volume (V2) was estimated in multiple brain regions: putamen, caudate, occipital cortex, prefrontal cortex, thalamus, and cerebellum. V2, representing the sum of specific and nonspecific binding (Ichise et al., 2001; Innis et al., 2007), was determined as the ratio of the average tracer concentration in the brain (C2) during steady state divided by the tracer concentration in plasma (C0). For the four regions of interest (ROD, namely, the prefrontal and occipital cortices, putamen, and thalamus, the NOP RO by LY2940094 (OR0 RO = V2RODrug/V2ROVND) was calculated based on the following equation:

\[ \frac{V_{2\text{RO}}}{V_{2\text{RO}}^\text{drug}} = \frac{V_{2\text{RO}}^\text{VND}}{V_{2\text{RO}}^\text{VND}} \]

where V2ROdrug, V2ROVND, and V2ROVND are the total distribution volume before (baseline) and after LY2940094 administration and nondisplaceable volume, respectively.

The nondisplaceable volume (V2ROVND) was estimated as the intercept of the Lassen plots (Lassen et al., 1995; Cunningham et al., 2010), where the difference between the baseline and blocked V2 values is plotted against baseline V2 for different regions of the brain.

Plasma Concentrations and Pharmacokinetics of LY2940094. Venous blood samples were collected in containers with an appropriate anticoagulant before and after each scan as well as serially over a 24-hour period. LY2940094
plasma concentrations were measured by a specific and validated LC-MS/MS method. The prevailing LY2940094 concentration during the scan was calculated as the average of the concentration before and after each scan. LY2940094 pharmacokinetic parameters were estimated using standard noncompartmental analysis methods.

**LY2940094 Plasma Concentration–NOP RO Relationship.** The relationship between the prevailing LY2940094 concentrations in plasma during the PET scan and the \(OE_{\text{RO}}\) values was characterized using a mixed-effect \(E_{\text{max}}\) model, where \(E_{\text{max}}\) was fixed to the theoretical value of 100%. The model estimated EC\(_{50}\) values and additive intersubject and within-subject variances using SAS version 9.2 (SAS Institute, Cary, NC).

**Results**

**LY2940094 NOP RO in Rat Brain.** LY2940094 potently, dose-dependently, and fully occupy the NOP receptor (Fig. 1A). In the time-course study, LY2940094 plasma and brain concentrations were generally parallel (Fig. 1B). Following its peak, RO followed an approximate linear decline (Fig. 1C), maintaining \(>50\%\) RO for at least 12 hours (by interpolation). A linear decline in RO is typical when drug levels decline exponentially, while the relationship between concentration and RO follows an Emax model. The Emax fit to the NOP RO vs. plasma LY2940094 levels (Fig. 1D) resulted in an EC\(_{50}\) \(\pm\) SE estimate of 5.75 \(\pm\) 1.25 ng/mL. RO was practically 100% between 5 minutes and 2 hours post dose, when LY2940094 was at its highest levels in plasma and brain tissue, approximately 99–206 ng/mL and 84–123 ng/g, respectively.

**LY2940094 Human Plasma Pharmacokinetics.** LY2940094 plasma levels started to rise after a 1- to 2-hour lag time that was quite variable across subjects (Fig. 2). Peak levels were achieved between 2 and 6 hours, followed by a biphasic decline: A rapid phase up to approximately 12 hours, followed by a slow terminal phase. The first postdose PET scan, which was a priori set at 2.5 hours to coincide with the peak drug levels, appears to have occurred too early in many subjects. The mismatch was most significant for the subjects taking the 4- and 40-mg doses.

The pharmacokinetic parameter estimates for LY2940094 are summarized in Table 1. C\(_{\text{max}}\) and area under the plasma concentration–time curve from 0 to 28.5 hours after dose administration (AUC\(_{0-28.5}\)) values appeared to increase approximately linearly as the LY2940094 dose increased, although the sample size was too small to test this hypothesis formally.

**Radiosynthesis and Measurement in Plasma of \([^{11}\text{C}]\text{NOP-1A.}\)**

Mean tracer specific activity of \([^{11}\text{C}]\text{NOP-1A}\) at the end of the synthesis was 5720 mCi/\(\mu\)mol (S.D. = 1600 mCi/\(\mu\)mol), and the mean mass injected was 1.57 \(\mu\)g (S.D. = 0.66 \(\mu\)g) with a radiochemical purity of 98.2\% (S.D. = 1.0\%). Parent \([^{11}\text{C}]\text{NOP-1A}\) typically formed 64\% of the total radioactivity in arterial blood samples.

\([^{11}\text{C}]\text{NOP-1A Time-Activity Curves and LY2940094 NOP RO.}\]

Sample PET images (Fig. 3) obtained from the same subject demonstrated the overall brain uptake of \([^{11}\text{C}]\text{NOP-1A}\) and its blockade by LY2940094.

Time-activity curves, presented as the ratio of radioactivity to the parent-related radioactivity in plasma, are shown in Supplemental Fig. 1. At baseline, the brain tracer uptake ratio levels in various regions of interest rapidly increased upon the regimen initiation to a peak level

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**Fig. 1.** NOP RO in rat hypothalamus after single oral dosing of LY2940094. (A) NOP RO 6 hours after dose administration as a function of dose, with \(E_{\text{max}}\) model fit (dotted line). (B) Plasma and brain LY2940094 concentrations over time after 1 mg/kg dose. (C) Time course of NOP RO and brain concentration after 1 mg/kg dose. (D) NOP RO as a function of time-matched LY2940094 plasma concentration, with \(E_{\text{max}}\) model fit (dotted line).
typically after 10 to 20 minutes (due to the bolus) and subsequently slightly decreased to steady-state levels. The steady-state level was highest in receptor-richer regions like the thalamus, occipital cortex, prefrontal cortex, and putamen, followed by the caudate and hippocampus; that in the cerebellar cortex was the lowest. Postdose scans revealed a reduction in radiotracer uptake (lower steady-state levels) that was more profound after higher LY2940094 doses and in peak time scans, which was consistent with the higher NOP blockade at higher plasma concentrations.

Supplemental Figure 2 shows several typical Lassen plots obtained. Linear regression fits were good, with $R^2$ ranging between 0.83 and 0.99, except for one scan after the 4-mg dose where the relationship was quite flat, indicating very low NOP occupancy.

Table 2 summarizes the mean NOP RO values by ROI, dose, and time point. LY2940094 exhibited dose-dependent target engagement of the NOP receptors after single oral doses (4–40 mg), exceeding 80% on average in the top dose levels. The NOP RO seemed to decrease moderately from the first to the second scan. The RO was similar across the different brain ROIs.

The $E_{\text{max}}$ model fitted the NOP RO-plasma concentration data well (Fig. 4). Table 3 summarizes the derived parameters from model fits.

**Safety.** LY2940094 was safe and well tolerated after single administrations of doses ranging from 4–40 mg. There were no deaths, serious AEs, or severe treatment-emergent adverse events (TEAEs). The overall incidence of TEAEs was 25%. TEAEs were mild in severity, with no relationship to dose, and were considered either unrelated or unlikely to be related to LY2940094. The most frequent TEAE was vessel puncture site pain, reported by two participants.

**Discussion**

We report here the results of our preclinical and clinical studies designed to demonstrate the NOP RO by the novel potent and selective NOP receptor antagonist LY2940094, which is currently under clinical development for the treatment of depression. Our studies relied on a pair of structurally similar NOP receptor tracers, LSN2810397 and [11C]NOP-1A, both containing spiroperidine, a common structural motif for NOP antagonists (Toledo et al., 2014) that is also shared by LY2940094. The studies examine the ability of rat NOP RO potency to predict human NOP RO potency. Additionally, these studies shed light on key attributes of LY2940094, including its systemic availability and pharmacokinetics, brain penetration, and the level of NOP RO in various regions of interest in the brain, providing a basis for dose selection for future development in target indications.

**Preclinical to Clinical Translation of NOP RO.** In translational drug development, it is desirable to use the RO results of a candidate drug in preclinical species for predicting its human behavior (Melhem, 2013). Cross-species potency differences could arise from differences in plasma protein binding, transport into the brain, and the degree of receptor homology. Good cross-species concordance has been found in multiple applications (Burns et al., 2007; Hostetler et al., 2013; Melhem, 2013). In others, significant differences existed but were explained by differences in protein binding. For example, the serotonin 1A (5-HT$_{1A}$) receptor antagonist (3R)-3-[di(cyclobutyl)amino]-8-fluoro-3,4-dihydro-2H-chromene-5-carboxamide (NAD-299) was shown to possess about 10-fold higher in vivo potency in monkeys than in humans, which could be accounted for by a 10-fold higher plasma free fraction (Andrée et al., 2003). In contrast, it was found that three structurally related inverse agonists for the GABA$_A$ receptors had $EC_{50}$ values in human that are a third to a fifth of those in rats and rhesus monkeys, which could not be

![Fig. 2. Individual LY2940094 plasma concentrations over time by dose in human subjects after single oral administration. Vertical lines are drawn to represent the timing of the two post-dose scans.](image-url)
accounted for by differences in protein binding or binding affinity (Atack et al., 2010; Eng et al., 2010). In our studies, the LY2940094 NOP RO EC$_{50}$ values in rats and humans were approximately 2-fold apart: 5.75 and 2.64–3.46 ng/ml, respectively. We assessed the binding affinity of LY2940094 in the membrane of Chinese hamster ovary cells expressing recombinant human NOP receptor and membranes isolated from whole rat brain with $K_i$ values of 0.11 and 0.71 nM, respectively (Statnick et al., 2016). The free fraction in plasma was also assessed in both species: 0.89% and 0.56%, respectively. Correcting the rat NOP RO EC$_{50}$ value by both factors gives a human-equivalent EC$_{50}$ of 0.56 ng/ml, overpredicting the human RO potency by 5-fold. A larger dataset for different receptor types and ligands would be needed before generalized translatability conclusions can be made. However, it is clear that a few-fold error in either direction should be expected. In this light, rats were reasonably predictive of human potency of NOP RO and can thus support using preclinical RO data to help design early clinical studies. Contributing factors to translation error include relatively imprecise in vitro methods as well as differences in the methods used to assess RO.

**Brain Penetration and RO Kinetics.** Multiple pieces of evidence suggest that LY2940094 equilibrates across the blood-brain barrier quickly, likely through passive diffusion. Data generated in our laboratories demonstrated that LY2940094 exhibited fast, passive permeability with high partitioning in Madin-Darby canine kidney cells, and was not a P-glycoprotein substrate (unpublished). Consistent with these results, plasma and brain concentrations were generally parallel in rats (Fig. 1B). Furthermore, during the model building of human data, we confirmed that the EC$_{50}$ values separately estimated after the first and second postdose scans were quite similar, suggesting a direct relationship between NOP RO and plasma concentrations with no hysteresis or lag, a phenomenon consistent with rapid equilibration between brain and plasma.

**LY2940094 Pharmacokinetics, NOP RO, and Dose Selection.** Overall, our PET study in healthy volunteers demonstrated brain penetration and specific target engagement of NOP receptors after single oral doses (4–40 mg) of LY2940094. The NOP RO increased with dose and concentration, and was generally higher at the first postdose PET scan (2.5 hours) compared with the second (26.5 hours). The NOP RO levels appeared similar at the 20- and 40-mg doses at approximately 80%. The RO remained high 26.5 hours after the 40-mg dose (above 70%). On a plasma LY2940094 concentration basis, LY2940094 potently occupied the NOP receptors, with EC$_{50}$ values consistent across ROIs, ranging between 2.64 and 3.46 ng/ml. Unlike the relationship with dose, the LY2940094 concentration–RO relationship clearly suggests that higher drug levels would produce an NOP RO approaching 100%, consistent with rat data. The discrepancy can be explained by

**TABLE 2**

Regional NOP receptor occupancy values (%) by time point and LY2940094 dose after single oral administration to human subjects

<table>
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<tr>
<th>Region of Interest</th>
<th>Scheduled postdose time (h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2.5 ((n=3))</td>
</tr>
<tr>
<td></td>
<td>26.5 ((n=3))</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>(\text{Mean}) 48.8</td>
</tr>
<tr>
<td></td>
<td>(\text{S.D.}) 42.6</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>(\text{Mean}) 44.1</td>
</tr>
<tr>
<td></td>
<td>(\text{S.D.}) 42.3</td>
</tr>
<tr>
<td>Putamen</td>
<td>(\text{Mean}) 51.3</td>
</tr>
<tr>
<td></td>
<td>(\text{S.D.}) 34.6</td>
</tr>
<tr>
<td>Thalamus</td>
<td>(\text{Mean}) 49.3</td>
</tr>
<tr>
<td></td>
<td>(\text{S.D.}) 40.4</td>
</tr>
</tbody>
</table>
LY2940094 plasma levels during the PET scans. LY2940094 absorption lagged by 1 to 2 hours and peaked 2 to 6 hours after oral dosing. Although the intent was for the first postdose scan to concur with the peak drug levels, the PET scan largely preceded the peak in most subjects, oftentimes starting at the beginning of the rising phase of the LY2940094–concentration relationship due to the significant absorption lag time (Fig. 2). Thus, the dose–RO relationship significantly underestimates the potency of LY2940094 at peak levels. By chance, the drug levels during the first postdose scan in the 20- and 40-mg dose groups were similar and hence the RO was similar (Table 2).

Given the apparent quick equilibration of LY2940094 and constant RO potency over time, NOP RO can be predicted from LY2940094 plasma concentration, using the established concentration–RO relationship. Given this relationship, we predict that the RO at mean observed peak concentration for the doses of 4, 10, 20, and 40 mg averaged over the four tested regions to be 73%, 88%, 93%, and 97%, respectively. Similarly, the plasma LY2940094 concentration 24 hours after dose administration is consistent with 28%, 49%, 65%, and 82%, respectively.

We measured RO after a single dose of LY2940094. However, the RO after multiple-dose administration is of greater interest for a drug intended for chronic administration. The data in this study alone are inadequate to make such prediction, as a multiple-dose pharmacokinetic examination would be necessary. However, it is clear from the concentration data up to 28.5 hours after dose administration that the terminal half-life of LY2940094 is significantly in excess of 24 hours; thus, a significant accumulation of 2-fold or higher is to be expected upon once-daily dosing of LY2940094. Accounting for 2-fold concentration accumulation, RO at trough levels would likely be in the vicinity of 90%. If NOP is similar to other G protein-coupled receptors such as dopamine D2 receptor antagonists as antipsychotic agents, a level of occupancy between 50% and 90% is associated with clinically efficacious doses (Grimwood and Hartig, 2009).

In rats, LY2940094 reversed the well-documented hypothermic effect of the NOP agonist 8-[[1S,3aS]-2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (Ro-64-6198) 2 hours after dose administration at 0.1 and 0.3 mg/kg, doses that are consistent with 50% to 80% NOP RO in our study (Toledo et al., 2014). Therefore, a 40-mg once-daily dose of LY2940094, producing NOP RO in excess of 80% throughout the dosing interval, is likely sufficient to test the therapeutic hypotheses of LY2940094, but a lower dose may also produce such a level of RO. Based on this information, a 40-mg daily dose of LY2940094 was recently used in a proof-of-concept study of major depressive disorder (Post et al., 2015), which was conducted after the studies mentioned in this report. In that study, an 8-week treatment with a once-daily 40-mg dose of LY2940094 provided some evidence of antidepressant effect (Post et al., 2015), further supporting the current hypothesis.

**Conclusion**

The [11C]NOP-1A tracer has proven useful for the assessment of the relationship between NOP RO, dose, and plasma concentration for the newly discovered NOP antagonist LY2940094. LY2940094 penetrates
the brain with apparent quick equilibration. Rat NOP RO potency was reasonably predictive of human potency. At oral doses that appeared safe and pharmacokinetics, a 40-mg

<table>
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<th>Region of Interest</th>
<th>Estimate</th>
<th>S.E.</th>
<th>95% CI</th>
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<tr>
<td>Prefrontal cortex</td>
<td>$E_{\text{MAX}}$</td>
<td>100% (Fixed)</td>
<td>2.94</td>
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<td>EC50 (ng/ml)</td>
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<td>$E_{\text{MAX}}$</td>
<td>100% (Fixed)</td>
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<td></td>
<td>EC50 (ng/ml)</td>
<td>5.42</td>
<td>1.18</td>
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<tr>
<td></td>
<td>Residual S.D.</td>
<td>15.42</td>
<td>3.44</td>
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Acknowledgments

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