Published in final edited form as:

Bioorg Med Chem. 2017 January 01; 25(1): 305–315. doi:10.1016/j.bmc.2016.10.035.

An evaluation of central penetration from a peripherally administered oxytocin receptor selective antagonist in nonhuman primates

Aaron L. Smith^{1,2,3}, Hasse Walum¹, Fawn Connor-Stroud⁴, Sara M. Freeman⁵, Kiyoshi Inoue¹, Lisa A. Parr¹, Mark M. Goodman^{1,2,3}, and Larry J. Young¹

¹Silvio O. Conte Center for Oxytocin and Social Cognition, Center for Translational Social Neuroscience, Department of Psychiatry and Behavioral Sciences, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road, Atlanta, GA 30329, USA

²Department of Radiology and Imaging Sciences, Emory University, Atlanta, Georgia 30322, USA

³Center for Systems Imaging, Emory University, 1841 Clifton Road NE, Atlanta, GA 30322, USA

⁴Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA

⁵California National Primate Research Center, University of California, Davis, CA 95616, USA

Abstract

The physiology of the oxytocin receptor has increasingly become a focus of scientific investigation due to its connection with social behavior and psychiatric disorders with impairments in social function. Experimental utilization of small molecule and peptide antagonists for the oxytocin receptor has played a role in deciphering these biological and social behavior connections in rodents. Described herein is the evaluation of a potent and selective oxytocin receptor antagonist, ALS-I-41, and details to consider for its use in nonhuman primate behavioral pharmacology experiments utilizing intranasal or intramuscular administration. The central nervous system penetration and rate of metabolism of ALS-I-41 was investigated via mass spectroscopy analysis of cerebrospinal fluid and plasma in the rhesus macaque after intranasal and intramuscular administration. Positron emission tomography was also utilized with [¹⁸F] ALS-I-41 in a macaque to verify observed central nervous system (CNS) penetration and to further evaluate the effects of administration rate on CNS penetration of Sprague-Dawley rats in comparison to previous studies.

Graphical Abstract

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Oxytocin Antagonist; Oxytocin Receptor; PET Imaging; Rhesus Macaque; Cerebrospinal Fluid

1. Introduction

As a hormone, oxytocin (OT) induces uterine myometrial contractions and postpartum mammary gland milk ejection by binding to the oxytocin receptor (OXTR) in these locations.[1] As a neuromodulator, OT modulates maternal nurturing, social recognition, social bonding, and empathy-related behavior by binding to OXTR in specific regions of the brain.[2–6] Our understanding of the neural mechanisms underlying OT's role in social bonding were facilitated by behavioral pharmacology experiments using selective peptide antagonists and by comparing the OXTR densities in brain autoradiograms of a socially monogamous species (*Microtus ochrogaster*) and promiscuous species (*Microtus montanus*) of voles.[7] The socially monogamous species was found to have a very high concentration of OXTR in the nucleus accumbens while the promiscuous species was found to have scarce OXTR density in this same region.[8, 9] As the nucleus accumbens is known for its role in cognitive processing of motivation, pleasure, reward, and addiction, the difference of OXTR density in these animal models emphasize its correlation to social bonding behavior.[10–15] Investigations into the localization and function of the OXTR system in human social behavior has since been an ongoing investigation in many facets of science including genetics, brain imaging, immunohistochemistry, *in situ* hybridization, and behavioral analysis.[16–19] The implications of the OT-OXTR neuromodulation system on human social behavior in clinical and typically developing populations has been a rapidly expanding contemporary focus in research which has resulted in both optimism as well as some criticism.[20–22]

The pharmacological viability of potent and selective OXTR antagonists was recognized upon the discovery of OT's physiological role in inducing uterine myometrial contractions; OXTR antagonists have been proven to function as tocolytic agents to prevent preterm labor.[23, 24] Further recognition of potential pharmacological use for an OXTR antagonist was acknowledged upon the discovery of OXTR in the hippocampus of a rat brain and the later correlations of OXTR to social behavior in various animal models including humans.[25–28] Hence, OXTR antagonists have been administered in pharmacological experiments for deciphering the role of OXTR signaling in behaviors and are potential pharmaceutical targets for treatment of specific disorders.[29–32] To investigate the OXTR neural functionality *in vivo* in a practical manner that does not involve invasive cranial surgery, the OXTR antagonist must be able to cross the blood brain barrier (BBB) when administered

peripherally. Without this functionality, the antagonist must be administered directly into the brain, making it impractical for translation to clinical use.

Very few OXTR antagonists have been reported which possess the ability to penetrate the BBB when administered peripherally. Additionally, limited data has been made available regarding their central nervous system (CNS) activity. The only peripherally administered OXTR antagonist which has been utilized for behavior pharmacology studies in the rhesus macaque (*Macaco mulatto*) is the Merck produced L-368,899.[33] Although there are several extensive reports regarding the peripheral pharmacokinetics of L-368,899 in various animal models due to its use in clinical trials, there are few documentations of pharmacokinetic details regarding its accumulation in the CNS. It was reported by Boccia et al that a small percentage of a dose of L-368,899 administered intravenously enters the cerebrospinal fluid (CSF) of a rhesus macaque and remains in the CSF for over 250 minutes post injection. They further evaluated the compound's accumulation in limbic system areas via mass spectrometry of homogenate tissue from specific regions. Our lab has investigated several OXTR antagonists (not all are yet published), some of which incorporated positron emitting isotopes into their structure and utilized positron emission tomography (PET) for brain penetration profiling. We reported a carbon-11 N-methylated derivative of L-368,899, but the slight difference in structure prevented the results from being directly comparable to L-368,899 itself.[34] However, it should be taken into consideration that N-methylated L-368,899 accumulated in the cynomolgus macaque's choroid plexus, the region of the brain responsible for producing CSF and filtering impurities out of CSF prior to brain entry. This could indicate CSF accumulation of the compound without achieving distribution throughout the entire brain. Although the report from Boccia et al did evaluate L-368,899 for brain distribution in postmortem tissue samples of the macaque, the analysis was performed via mass spectrometry on tissue homogenate extractions. The data obtained from this methodology had increased experimental variables which could have skewed the data (there were no reported experimental controls with standards concurrently reported and the sample sizes were very small). Brain tissue homogenates also contain capillaries which may give false positive measurements, especially when the amounts are in the picogram range.

Intranasal (IN) administration of OT has been utilized quite regularly as a potential means to reach the brain. Indeed, elevated levels of OT have been detected in the CSF after IN administration of OT in humans as well as the rhesus macaque.[35] Various mechanisms by which OT increases in the CSF after IN administration have been hypothesized, yet none of the mechanisms have been proven experimentally.[36–38] Copious investigations of IN administration of OT have reported effects in both normal subjects and subjects having social behavior deficits.[22, 39–44] Due to OT being endogenously produced in the hypothalamus and distributed throughout the CNS, it is disputable that measured elevations of central OT concentrations post IN administration derived from the IN dose itself.[45] The mechanisms which induce hypothalamic production and distribution of OT are not completely understood, and it can be hypothesized that an increase of peripheral OT signals hypothalamic release of OT into the CNS.[46] Surprisingly, there has been limited research investigating the effects of IN administration of an OXTR antagonist.

The OXTR is one of a growing list of identified G-protein coupled receptors (GPCR), a list that has now grown to nearly 3000. The probability of a molecule antagonizing only one GPCR without agonizing, antagonizing, or allosterically modulating another GPCR is very low. Therefore, when evaluating the behavior effects of a specific GPCR using a molecule of known affinity and functionality, having or building a knowledge base of a specific compound's potential off-target binding to other GPCRs is essential. In consideration of the high probability of off-target binding, it would be beneficial to investigate behavioral effects using an alternative molecule of entirely different structure to confirm observations. This is especially true when the evaluation involves a high level of experimental variables as is the case with behavioral assessment. Here, we build upon the knowledge base of a previously reported OXTR antagonist (ALS-I-41, Figure 1) to evaluate its ability to breach the blood brain barrier in macaque models through aerosolized IN, intramuscular (IM), and IV routes of administration.[47] Plasma and CSF samples were collected at specific time points post IN and IM administration and analyzed via liquid chromatography mass spectrometry (LCMS). A PET scan was performed after IV administration of [^{18}F]ALS-I-41. The use of PET with [^{18}F]ALS-I-41 was also performed in Sprague-Dawley rats to further evaluate metabolism and brain penetration differences that may occur with bolus IV, slow infusion IV, and IM routes of administration.

2. Material and methods

2.1 General

The synthesis and radiolabeling synthesis of ALS-I-41 and [^{18}F]ALS-I-41 was performed as previously reported.[47] Propylene glycol, United States pharmacopeia grade, was purchased from Fisher Scientific. The methodology for LCMS analysis of ALS-I-41 in CSF and plasma was first developed on an Advion CMS expression and carried over to an AB Sciex 6500 Triple Quadrupole with a Shimadzu Nexera X2 Autosampler and LC tower. Nonhuman primate PET images of the brain region were obtained with a Siemens Focus 220, the PET isotope, and a 10 min transmission scan with a cobalt-57 source for attenuation correction. The Focus 220 has a 22 cm gantry containing a full ring of 168 LSO detector blocks with a 12 x 12 array having an axial field of view of 7.7 cm which provide 24,192 detector elements and a coincidence detection timing window of 6 ns. Resolution from reconstruction is approximately 1.3 mm in the transaxial planes at the center of the field of view. PET images of Sprague-Dawley rats were obtained using a Siemens Inveon dedicated PET tomograph, the PET isotope, and a 7 min transmission scan using a cobalt-57 source for attenuation correction. Scans were performed in a 12.5 cm field of view starting with the nose of the rodent. Spatial resolution of reconstructed images are approximately 1.4 mm. More details about the functionality of the Inveon scanner is published in Bao et al.

All procedures used in this study were reviewed and approved by the Institutional and Animal Use Committee. It is the policy of Emory University to adhere conscientiously to all of the humane standards set by the Animal Welfare Act (United States Department of Agriculture/USDA), the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Standards of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), whose certification is regarded as the

gold seal of approval for laboratory animal care, and the policies of the Emory University Board of Trustees.

2.2 CSF and Plasma Extraction Experiments

2.2.1 Dose Preparation for Intranasal and Intramuscular Administration—Dose solutions were prepared in a low (120 µg/ml) or high (6.15 mg/ml) concentrated solution in 10 ml of propylene glycol. Propylene glycol was necessary to ensure adequate solubility of the compound. Into a sterile empty vial was weighed 1.2 or 60 mg of ALS-I-41 and 10 ml propylene glycol was then injected. The mixture was vortexed until all solids were dissolved. Using pressurized argon, the dose was then passed through a sterilized line equipped with 1 micron and 0.45 micron sterilized filters into a sealed sterile vial.

2.2.2 Procedure for Dose Administration and Fluid Collection in a Rhesus

Macaque—Four male rhesus macaques born at Yerkes National Primate Research Center were used for this study. The monkeys were five years old and weighed 9–13 kg at the time of each trial (average weight of 11.4 kg for all trials). The monkeys were anesthetized directly from their home cage using an IM dose of Telazol (4–5 mg/kg) and taken to a treatment room. Anesthesia was maintained with propofol (1–2mg/kg) at a concentration of 10 mg/ml. The propofol was diluted using a Lactated Ringer's solution at a concentration of 1 mg/ml and delivered IV at a drip rate of 10 mg/kg/hr. Heart rate, respiration and pulse oxygen saturation were monitored throughout the procedure. The lumbar region of the subject was shaved and prepped with an iodine solution for CSF extraction.

For IN delivery, the subject was fitted with a face mask connected to a pediatric nebulizer and the aerosolized dose was administered for 8 minutes. This procedure was similar to that previously described by Modi et al, but the time of administration was extended due to the viscosity of propylene glycol.[37] Two dose levels (a high dose and a low dose) were administered IN. The average low dose administered (n=8) was 205 µg. The average high dose administered (n=2) was 7.69 mg. Dose amounts were calculated from volume remaining in the nebulizer apparatus. For IM administration, a 6.15 mg dose (n=2) was injected into the inner thigh muscle of the subject, the low dose was not used for IM administration.

Only two baseline CSF samples were acquired to confirm there was no mass peak equivalent to ALS-I-41 detected on our LCMS analysis prior to administration. CSF samples were taken at 60 and 120 min post nebulization or injection and were placed immediately in dry ice and stored in a –80 C freezer until they were thawed for analysis. Repeat studies on the same monkey were not performed until at least two months of time had passed. CSF samples were collected by passive drip through a 22 G needle inserted into the lumbar subarachnoid space for each sample as previously described by Modi et al.[37] Contaminated CSF samples (samples with a pink tinge indicating red blood cells) were not used for data analysis for this study. Three attempts to obtain a clean sample were made for each time point. If a clean sample could not be obtained, that time sample was not collected during that session and the treatment was re-administered at a later date to obtain any missed samples for each subject.

Blood extractions were acquired from the saphenous vein. Prior to any compound delivery, baseline blood samples were collected. Blood samples were taken at 5, 15, 30, 60, and 120 min, placed on ice until the end of the study, and then spun for 10 minutes at 10,000 rpm. Plasma was separated from the red blood cells and stored at -80°C until they were thawed for analysis.

2.2.3 Plasma and CSF Sample Preparation and Analysis—CSF samples were analyzed without further preparation. Preparation of extracted plasma samples was conducted in a 0.5 ml 96 well plate. To each well of the plate was added 100 μl water, 25 μl of the internal standard diluted 100 times by mass in acetonitrile, and 100 μl of the plasma sample. The samples were mixed in the well by pipetting 8–10 times. Using the same pipette tips used for mixing, the diluted samples were transferred to a solid-liquid phase extraction set atop a 2.0 ml 96-well plate. The samples were loaded onto the columns by pulse vacuum for 5 seconds. After resting for 5 minutes on the sorbent bed, 1 ml ethyl acetate was added. After another 5 minutes, vacuum was applied and the samples were collected in the 2.0 ml 96-well plate. The samples were then concentrated using a Biotage turbovap 96 at 30 PSI nitrogen pressure at 37°C and then diluted with 100 μl of 70:30:0.1 water/ methanol/ formic acid. Samples were then measured for ALS-I-41 concentration in duplicate using an AB Sciex 6500 Triple Quadrupole mass spectrometer equipped with a Shimadzu Nexera X2 autosampler and LC tower. Injected samples passed over a Phenomenex Kinetex 2.6 μm C18 100 Å bed LC column with a 2.1mm diameter and a 500 mm length eluted with 70:30:0.1 water/ methanol/ formic acid at 0.6 ml/ min. The column temperature was 40.0°C and had a backpressure of 5200 psi. Injection volume was 20 μl . The extraction protocol was tested with rhesus plasma samples of known concentrations and recovery rate was approximately 86%.

Acquired samples from subjects were ran concurrently with prepared samples of an internal standard of ALS-II-69, a compound of similar structure as ALS-I-41, but with a significantly different retention time on the LC column.[48] Samples of ALS-I-41 were prepared and analyzed in rhesus plasma and artificial CSF in concentrations ranging 0.8 to 10 pg/ml to generate a calibration curve. The detection limit of ALS-I-41 in CSF and plasma was approximately 1 and 8 pg/ml respectively.

2.3 PET Imaging

2.3.1 PET Scan Acquisition of a Macaque—A male cynomolgus macaque (*Macaca fascicularis*) weighing 8.1 kg was acquired from Yerkes National Primate Research Center for this study (n=1). This cynomolgus monkey was chosen instead of a rhesus macaque due to the animal already having an MRI on file to co-register with PET. The monkey was anesthetized with Telazol (3 mg/kg; i.m.) and maintained on a 1–2% isoflurane in pure oxygen mixture while intubated and placed on a ventilator throughout the imaging procedure. Heart rate, temperature, respiration, and pulse oxygen saturation were monitored continuously after anesthesia was established. A 15 minute transmission scan was performed using a germanium-68 point source to provide attenuation correction. Approximately 5 mCi of [^{18}F]ALS-I-41 was then administered via an IV bolus at the start of the emission scan and scanning continued for 2 hours. Reconstructed images were processed using Seimens

ASIPRO software. Volumes of interest (VOI) were acquired by outlining regions of interest on several slices to generate 3-D data points on time-activity curves (TAC). TAC's were generated by the software in nCi/cubic centimeter and converted into standard uptake values (SUV) by dividing the volume of interest for each point by the radioactivity injected multiplied by the body weight.

2.2.4 Procedures for PET Scan Acquisitions of Sprague-Dawley Rats—Female Sprague-Dawley rats (200 – 330g) acquired from Charles River were placed in a chamber with 4–5% isoflurane in oxygen at a flow rate of 1000 ml/min to induce anesthesia. Anesthesia was maintained continuously until the imaging procedure was finished at 1 – 2% isoflurane in oxygen at a flow rate of 500 – 1000 ml/min using Kopf stereotaxic nose cones with outlets vented thorough a weighed carbon filter. The rat's vital signs were monitored using the Bio-Vet (M2M Imaging) hardware and software monitoring system which includes a resistive Bair Hugger patient warmer with temperature probe feedback, a pressure sensor for monitoring respiration rate, and 3 EKG leads for measuring heart rate. Rats receiving IV continuous infusion (n=2) of [¹⁸F]ALS-I-41 were affixed with a tail vein catheter. Rats were placed on a carbon fiber bed in the prone position for imaging. An eye gel was used to protect the cornea from drying.

Rats receiving an IM injection (n=3) were injected with 340–390 μ Ci of [¹⁸F]ALS-I-41 in 100 μ l just above the knee of the hind leg as is recommended to be the optimal volume by Felix et al on the Charles River website files (http://www.criver.com/files/pdfs/nonsource/act_2014_max_vol_injected_intramuscularly_rats.aspx). Scanning began at 15 minutes post injection to allow for the radioligand to dissipate from the tissue. The scan time was 2 hours.

Rats receiving a continuous IV infusion were administered 250–310 μ Ci [¹⁸F]ALS-I-41 by filling a 300 μ l line with 550 – 600 μ Ci [¹⁸F]ALS-I-41, connecting one end of the line to their tail vein catheter and the other end to a syringe filled with 10% ethanol in saline (the dose solvent) on a mechanical syringe pump. The syringe pump infused the dose at a rate of 30 μ l/min for 30 minutes. Scanning was started at one minute after the infusion began and continued for 2 hours. All reconstructed images were then processed with ASIPRO software as described in section 2.2.3 except for the conversion to SUV's. For clarity of the data, the mean nCi/cc value was used.

3. Results and Discussion

3.1 Plasma and Cerebrospinal Fluid Concentrations of ALS-I-41

The measured concentrations of ALS-I-41 in plasma and CSF extractions are shown in Figure 2. The initial target dose for IN administration of ALS-I-41 was 234 μ g. This amount was chosen because it is ten times the molar equivalent of 24 international units of OT, an amount previously used in clinical trials investigating the effects of IN OT.[35] A total of 8 trials were performed at this low dose, with an average dose of 202 μ g administered. Of the 8 trials, CSF samples containing blood were omitted from the data, leaving four data points for each time point. There were no omissions of any plasma data. The resulting concentrations from the low dose IN were extremely low with an average peak of 1 ng/ml in plasma and 7 pg/ml in CSF. After observing such low concentrations in the plasma and CSF

from the low dose IN administration, the IN target dose was increased to 7.5 mg using a 6.15 mg/ml solution of ALS-I-41. This much higher IN dose resulted in 150 fold increase in plasma concentrations and a 14 fold increase in CSF concentration in comparison to the low dose. To investigate an alternative practical method of administration in the field, intramuscular (IM) administration of 6.15 mg was investigated. The IM injection resulted in the highest fluid concentrations with 5 fold concentration increases over the high dose IN administration for both plasma and CSF. Two trials were performed with the high dose IN and two trials were performed with the IM dose. All CSF samples obtained for the high dose IN and IM studies were free of blood contamination; no data was omitted from these trials. Table 1 summarizes the dose information, the corresponding maximum plasma and CSF concentrations, and the times in which the maximum concentrations occurred.

The use of a pediatric nebulizer for IN administration likely accounted for a substantial portion of the 5 fold concentration deviation between IM and high dose IN administration as unmeasured amounts of the IN doses were lost in air during the 8 minute nebulization period. The nebulizer, as opposed to a direct nasal spray administration, was used in this study because the monkeys can be trained to use it, it has been found to be more effective, and we are interested in practical methodologies for behavioral assessments.[37] Although the fluid concentrations were lower than we had hoped in the nebulizer trials, the acquired data does provide useful information. The IN administration plasma concentrations allowed for a calculation of the elimination rate (k_e) of ALS-I-41 in rhesus plasma using the equation $k_e = \ln C_1 - \ln C_2 / (t_2 - t_1)$ with the maximum and minimum measured concentrations (C_1 and C_2 respectively) and the corresponding times. Applying this equation to the low and high IN plasma data resulted in a k_e of 0.024/min. The k_e was confirmed by obtaining the slope from a linear regression on the semi-log plot of four plasma concentration data points for both the low and high dose IN data. Using this k_e value, the half-life of ALS-I-41 in plasma was calculated to be $0.693/k_e = 29$ minutes. The same calculations, when applied to the high dose IN CSF data, provides a k_e for ALS-I-41 in the CSF of 0.007/min and a half-life in the CSF of 96 minutes. As there are only two valid CSF data points for each trial (due to the difficulty of obtaining multiple CSF samples in one session) the reliability of the k_e value and half-life for the CSF is low.

The time of maximum concentrations in the plasma and CSF varied, as expected, for the IM and IN administrations. The IM administration resulted in a gradual increase in plasma concentrations with maximum plasma concentration occurring 45 minutes later than IN administration. The delay was attributed to additional time required for the dose to pass through the myofibril matrix into the bloodstream. As was also expected, the rate of diffusion through the myofibril matrix varied per injection (due to the variance of the depth of the injection into the tissue) which resulted in larger concentration deviations at the various time points. Maximum concentration in CSF was at the 2 hour mark for IM whereas the high dose IN peaked at one hour. Considering the estimated CSF half-life of 96 minutes and the variance in the diffusion through the myofibril matrix, it is possible the maximum CSF concentration after an IM injection can occur after 120 minutes.

As reported by Modi et al, aerosolized IN delivery of 24 IU of OT increased CSF OT levels in rhesus macaques from a baseline level of 30 pg/ml to approximately 70 pg/ml at 60 and

120 min post administration.[37] Assuming a macaque CSF total volume of 10 ml, the CSF concentrations increased from a baseline of 3 pM to post administration levels of 7 pM. Using the same calculation in a similar study by Freeman et al in which a maximum of 5 IU/kg OT was administered via IV and IN, the maximum reported CSF increase reached the 22 pM range for IV but there was no significant change from the IN nasal spray.[38] It should be noted that the IN administration method used in the Freeman et al study was slightly different from our approach; a nasal “spray” was used instead of a continuous aerosolized mist from a pediatric nebulizer. A deep inhalation would likely be required from the subject during the spraying into the nasal cavity to achieve the same results as the aerosolized IN administration. In comparison to the antagonist administered in this study, the high dose aerosolized IN administration of ALS-I-41 resulted in a maximum CSF concentration of 16 pM at the 60 min time point. Likewise, the IM administration resulted in a max concentration of 85 pM at 120 min post administration. Considering the K_i of ALS-I-41 for human OXTR (16 nM) was determined *in vitro* by displacing [^3H]OT at a concentration of 2 nM, a starting point for adequate dosing for behavior responses could be deduced from these number using baseline concentration measurements of OT. Although the *in vitro* conditions are quite different than *in vivo* conditions, a CSF concentration approximately 8 times greater than that of OT would theoretically be required to displace OT from the OXTR based on the *in vitro* K_i determination. The measured baseline OT levels (3 pM) from the Modi et al and Freeman et al experiments infer that a concentration greater than 24 pM would be necessary to displace endogenous OT and elicit behavioral effects. Furthermore, to block the effects of 24 IU OT administered aerosolized IN to a macaque, a concentration greater than 56 pM would be necessary. Therefore, the IM administration of 6.15 mg ALS-I-41 appears to be an adequate method of administration to achieve CSF concentrations high enough to elicit or block a behavior responses of the OT system. Although it should be considered that peripheral OXTR would be also be subjected to a blocking effect. These results are summarized in Table 2.

Dosing estimates of ALS-I-41 for humans can also be deduced from the data in this study and previously reported work. In a study reported by Striepen et al, an IN administration of 24 IU of OT increased OT levels in human CSF from a baseline of 20 pg/ml to 30 pg/ml at 75 minutes post administration.[35] Assuming a 135 ml total CSF volume, this equates to a concentration rise from 0.15 pM to 0.22 pM. Therefore, the target CSF concentration of ALS-I-41 for baseline OT levels would be 1.2pM (95 pg/ml CSF) to elicit a neural OXTR antagonistic behavior response. To block the effects of IN administration of 24 IU OT, a target CSF concentration of ALS-I-41 would be 1.8pM (140 pg/ml CSF). Again, the IN administration method used in the Streipens et al study was a nasal spray and not a continuous aerosolized mist from a pediatric nebulizer. However, the human subjects in this case were instructed to inhale deeply during spraying into the nasal cavity. Although this difference in methodology would not alter OT levels in the CSF prior to IN OT administration, Modi et al results and discussion suggest it still could have resulted in a lower post-administration level. However, for the antagonist ALS-I-41, as the data revealed IM to be the most efficient method of administration to reach the CSF, IN administration is not required.

Importantly, the amount of IN OT detected in the CSF from in the Stiepens et al, Modi et al, and Freeman et al publications is theoretically not enough to completely saturate the OXTR assuming 135 ml CSF volume in humans and 10 ml of CSF volume in the macaque. Similarly, for this study, the concentration of ALS-I-41 is too low to saturate the OXTR. However, the measured amounts in CSF from all of these experiments do not take into consideration OT or ALS-I-41 that have been taken up by the OXTR receptors or other biological entities within the brain at the time of measurement. Furthermore, both OT and ALS-I-41 have a much longer half-life in CSF than plasma and the incubation times used for *in vitro* measurements. The prolonged *in vivo* exposure of these chemicals to the neural OXTR is likely responsible for enabling behavioral effects. As the next section will demonstrate, the concentration of these chemicals, especially ALS-I-41, vary throughout the brain at different times. Further microdialysis experiments may aid in clarifying the real-time concentrations of OT or ALS-I-41 after administration at specific regions. As previously mentioned, OT is endogenous and there is no discrepancy from exogenously administered OT; any increases of OT in the CSF after exogenous administration of OT can only be assumed to be exogenous OT until further experimental data is obtained. We have shown here that the completely exogenous OXTR antagonist, ALS-I-41, can reach the brain in comparable amounts to what was measured after IN administered OT and it is retained within the brain for over 2 hours. We have also shown the antagonist does not require IN administration to achieve these amounts. Considering the various studies reporting behavioral responses from such a low dose of IN OT, it can be hypothesized that the OXTR antagonist ALS-I-41 could elicit an antagonistic behavioral response at the dose levels described herein as well as block behavioral effects derived from IN administration of 24 IU OT. However, a combination of peripheral administration of ALS-I-41 and central OT administration using electrophysiological recordings in OT sensitive brain regions would be ideal for determining the true efficacy of peripherally administered antagonist to probe central OT function.

3.2 PET Imaging

3.2.1 PET Imaging of [¹⁸F]ALS-I-41 in a Cynomolgus Monkey—To visualize the distribution of ALS-I-41 in the living brain of a macaque, a PET scan was performed in a cynomolgus macaque for 2 hours after administration of 5 mCi of [¹⁸F]ALS-I-41 via IV administration. The time-activity curves (TAC's) generated from the scan are provided in Figure 3. The TAC of the tissue from the neck muscles and whole brain (Figure 3a) indicate that only a fraction of the dose reaches the brain; [¹⁸F]ALS-I-41 was not distributed equally between central and peripheral regions. These results confirm what was observed in section 3.1. Interestingly, the TACs of various brain regions (Figure 3b) indicate unequal distribution of [¹⁸F]ALS-I-41 in the brain throughout the scan. The cerebellum, midbrain and brain stem had the strongest signals followed by the temporal and frontal lobes. All of these regions had stronger uptake than the average uptake of the entire brain. The occipital and parietal lobes as well as the thalamic region (including the corpus callosum, fornix, and lateral ventricles) had the lowest uptake. Representative images from the scan are shown in Figure 4 with arrows indicating the accumulations in the cerebellum, midbrain/brain stem, frontal and temporal lobes.

In comparison to previously reported autoradiography and in situ hybridization studies with rhesus macaque brain slices, [^{18}F]ALS-I-41 was more concentrated within the same regions shown to contain OXTR (nucleus basalis of Meynert, pedunculopontine tegmental nucleus, the superficial gray layer of the superior colliculus, the trapezoid body, and the ventromedial hypothalamus).[49, 50] While there was little accumulation of [^{18}F]ALS-I-41 in the thalamus region, there was a higher concentration of [^{18}F]ALS-I-41 in the regions containing the central amygdala, anterior cingulate cortex, entorhinal, claustrum, subiculum, and parasubiculum. These regions were all found to display an autoradiography signal from [^{125}I]ornithine vasotocin, but are not believed to be sites of OXTR synthesis based on in situ hybridization results. Nevertheless, the generally low levels of radiotracer in the brain, and the overall low OXTR densities in the macaques prevented OXTR densities to be reliably quantified. The important aspect of the study was the detection of [^{18}F]ALS-I-41 in regions known to contain OXTR.

What was not documented previously and is noted here is the accumulation of [^{18}F]ALS-I-41 in the cerebellar regions. In situ hybridization data of the cerebellar regions was not included in the previous report (referenced above) because the granular layers of the cerebellum have been known to display non-specific binding for *in situ* hybridization experiments. Although a signal was observed with the in situ hybridization experiment, it was not confirmed and, therefore, not included in the previous publication. Figure 5 shows examples of OXTR *in situ* hybridization results of the cerebellum (obtained with the same methodology as the previous publication) with the red arrow indicating the observed unconfirmed signal.[49] However, because of background variability in sense strand controls across several experiments, we are not confident that the in situ hybridization signal is truly reflective of OXTR synthesis. In combination with the PET data, future studies will need to determine if these signals in the cerebellar region are indeed OXTR or false positives.

Interestingly, there is a stark contrast between observed accumulation of [^{18}F]ALS-I-41 in the various regions of the limbic system. This can easily be seen by comparing the accumulation densities in the thalamic region and temporal lobe. While the distribution of the tracer throughout the entire brain peaked and was nearly equally distributed at approximately 15–30 minutes into the scan (Figure 6), very little remained in the thalamic region at the end of the scan. However, the temporal lobe contained approximately 75% of its peak signal at the end of the scan. While the macaque OXTR densities reported in the temporal lobe region are rather low based on autoradiography and *in situ* hybridization results, there are confirmed reports of OXTR in the temporal lobe region while there are no reports of OXTR in the thalamus. Also, autoradiography is thought not to show signals from terminal fields of OXTR synthesizing brain regions.[51] Although the signal acquired from [^{18}F]ALS-I-41 did accumulate more in regions where OXTR has been reported and the CSF data suggests the molecule does indeed penetrate to the CNS, it is to be determined if this accumulation is significant and related to OXTR densities. In the macaque model, [^{18}F]ALS-I-41 is the only PET tracer evaluated that has shown an unevenly distributed signal in the brain. Therefore, there is limited choice in selecting a molecule capable of blocking a signal from the OXTR. Consideration in administering L-368,899 as a blocking agent was given, but we were first interested in possibly improving the observed signal as well as investigating the production of L-368,899 as a PET tracer. The following study in rat

models was performed to evaluate alternative administrations of [^{18}F]ALSI-41 for improving brain signal as well as further evaluating the molecule as viable for use in behavior studies in rodents.

3.3 PET Imaging of Sprague-Dawley Rats

A 30 minute continuous infusion IV injection and an IM bolus injection studies were performed with [^{18}F]ALS-I-41 in Sprague-Dawley rats and compared to previously obtained IV bolus injection.[47] Graphs of the TACs derived from the three PET imaging studies are shown in Figure 7 and include data from the whole brain, muscle tissue from the arm, and skull. We had previously investigated [^{18}F]ALS-I-41 via IV bolus injection and, based on the TACs and images, thought the compound was not a brain penetrant in the rat. The TAC curve derived from the IV bolus does show a slow increase of activity in the brain from the 15 minute time point through 90 minutes, but it also showed an increase in the bone uptake as well (there was no significant bone uptake detected in the monkey model). The bone uptake suggests the molecule is losing its fluorine to metabolism and creating fluoride which is capable of penetrating to the brain, but is generally taken up in the bone. The image generated from the IV bolus also suggested very little uptake in the brain. The appearance of low uptake (even if summed at the latter portion of the scan) is believed to be due to the periphery accumulating twice the amount of activity as the brain via the IV bolus injection modality. In comparison, the IM and 30-minute continuous IV infusion data had less peripheral uptake and, therefore, the contrast between the brain and periphery enable the signal within the brain to be better visualized than in the IV bolus injection (Figure 8). The TAC from the continuous infusion scans show that brain uptake increased while bone uptake remained relatively constant after the entirety of the dose was administered (at the 30 minute point). Considering fluorine is rapidly trapped in the bone, the signal in the rat brain is most likely not entirely from defluorination, but could contain the parent compound as well as other metabolites. When considering published data showing little to no accumulation of [^{18}F]sodium fluoride in the rat brain, the latter seems the most probable.[52] Nevertheless, the rapid uptake in the bone does suggest the compound is rapidly metabolized on first pass in the rat. As there was no significant bone uptake in the macaque scan, the method of metabolism is clearly different than the macaque. The TAC from the IM injection as well as the image itself (very prominent skull uptake in the image) indicates more bone uptake occurs using an IM injection. The extended exposure in the myofibril matrix did not promote stability of the compound.

Notably, the TAC of each injection method for the brain was similar. There was no reduction in activity from the brain in any of the scans once it reached its maximum and the maximum amounts within the brain were all close in range. There was no noticeable clearance of the radioactivity once within the brain during the scanning timeframes, resembling what was found when investigating the same molecule in a marmoset model. Although radioactivity was detected in the rat brain, CNS penetration of [^{18}F]ALS-I-41 was not confirmed via CSF extraction as was performed with the macaques. Due to brain penetration varying between different animal models, further confirmation via rat CSF extraction will be required to determine if the uptake observed in the rat brain was a metabolite or the parent compound. This report confirms the variance in brain penetration of [^{18}F]ALS-I-41 between animal

models as, previously reported, no significant brain penetration of [^{18}F]ALS-I-41 was detected in titi monkey models.[53] However, the data from the various rat scans does lead to the hypothesis that if a 30 minute continuous IV infusion of [^{18}F]ALS-I-41 is performed with a macaque model then an image with improved resolution may be obtained.

4. Conclusions

The small molecule OXTR antagonist ALS-I-41 was discovered to be a mild brain penetrant in the rhesus macaque via CSF extraction and PET imaging. The PET imaging data acquired with [^{18}F]ALS-I-41 in a macaque model demonstrated, for the first time, accumulation of an OXTR selective radiotracer in brain regions known to contain OXTR. The resolution of the images, however, did not enable adequate quantification of receptor density. PET imaging data from different injection methods of [^{18}F]ALS-I-41 in rat models demonstrated an improved brain resolution by performing a 30 minute continuous IV infusion. The small molecule, ALS-I-41, is a viable tool for investigating the behavior effects of an OXTR antagonist in the macaque utilizing the dose amounts described herein. However, the ability of ALS-I-41 to act as a brain penetrant varies between animal models as does its route of metabolism. Therefore, confirmation of brain penetration must be performed for each animal model investigated with it.

Acknowledgments

This research was supported by NIH grant 1P50MH100023 to LJY. Additional funding was provided by NIH OD P51OD11132 to YNPRC.

References

1. Burbach, JPH.; Young, LJ.; Russel, JA. Knobil and Neill's Physiology of Reproduction, Chapter 58, Oxytocin: Synthesis, Secretion, and Reproductive Functions. 3. Elsevier Academic Press; 2006.
2. Numan M, Young LJ. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. *Horm Behav.* 2016; 77:98–112. [PubMed: 26062432]
3. Rilling JK, Young LJ. The biology of mammalian parenting and its effect on offspring social development. *Science.* 2014; 345:771–776. [PubMed: 25124431]
4. Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J Neurosci.* 2001; 21:8278–8285. [PubMed: 11588199]
5. Johnson ZV, Young LJ. Neurobiological mechanisms of social attachment and pair bonding. *Curr Opin Behav Sci.* 2015; 3:38–44. [PubMed: 26146650]
6. Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FB, Young LJ. Oxytocin-dependent consolation behavior in rodents. *Science.* 2016; 351:375–378. [PubMed: 26798013]
7. Johnson ZV, Walum H, Jamal YA, Xiao Y, Keebaugh AC, Inoue K, Young LJ. Central oxytocin receptors mediate mating-induced partner preferences and enhance correlated activation across forebrain nuclei in male prairie voles. *Horm Behav.* 2016; 79:8–17. [PubMed: 26643557]
8. Young LJ, Wang ZX. The neurobiology of pair bonding. *Nature Neuroscience.* 2004; 7:1048–1054. [PubMed: 15452576]
9. Insel TR, Shapiro LE. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Natl Acad Sci U S A.* 1992; 89:5981–5985. [PubMed: 1321430]
10. Chick CF. Reward Processing in the Adolescent Brain: Individual Differences and Relation to Risk Taking. *J Neurosci.* 2015; 35:13539–13541. [PubMed: 26446208]

11. Bergamini G, Sigrist H, Ferger B, Singewald N, Seifritz E, Pryce CR. Depletion of nucleus accumbens dopamine leads to impaired reward and aversion processing in mice: Relevance to motivation pathologies. *Neuropharmacology*. 2016; 109:306–319. [PubMed: 27036890]
12. Mueller K, Jech R, Schroeter ML. Deep-brain stimulation for Parkinson's disease. *The New England journal of medicine*. 2013; 368:482–483. [PubMed: 23363513]
13. Smith KS, Berridge KC, Aldridge JW. Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci U S A*. 2011; 108:E255–264. [PubMed: 21670308]
14. Bosch OJ, Dabrowska J, Modi ME, Johnson ZV, Keebaugh AC, Barrett CE, Ahern TH, Guo J, Grinevich V, Rainnie DG, Neumann ID, Young LJ. Oxytocin in the nucleus accumbens shell reverses CRFR2-evoked passive stress-coping after partner loss in monogamous male prairie voles. *Psychoneuroendocrinology*. 2016; 64:66–78. [PubMed: 26615473]
15. King LB, Walum H, Inoue K, Eyrich NW, Young LJ. Variation in the Oxytocin Receptor Gene Predicts Brain Region-Specific Expression and Social Attachment. *Biol Psychiatry*. 2016; 80:160–169. [PubMed: 26893121]
16. Skuse DH, Lori A, Cubells JF, Lee I, Conneely KN, Puura K, Lehtimäki T, Binder EB, Young LJ. Common polymorphism in the oxytocin receptor gene (OXTR) is associated with human social recognition skills. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:1987–1992. [PubMed: 24367110]
17. Walum H, Lichtenstein P, Neiderhiser JM, Reiss D, Ganiban JM, Spotts EL, Pedersen NL, Ankarsater H, Larsson H, Westberg L. Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biol Psychiatry*. 2012; 71:419–426. [PubMed: 22015110]
18. Rilling JK, DeMarco AC, Hackett PD, Thompson R, Ditzen B, Patel R, Pagnoni G. Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. *Psychoneuroendocrinology*. 37:447–461. [PubMed: 21840129]
19. Scheele D, Wille A, Kendrick KM, Stoffel-Wagner B, Becker B, Gunturkun O, Maier W, Hurlmann R. Oxytocin enhances brain reward system responses in men viewing the face of their female partner. *Proc Natl Acad Sci U S A*. 2013; 110:20308–20313. [PubMed: 24277856]
20. Shamay-Tsoory S, Young LJ. Understanding the Oxytocin System and Its Relevance to Psychiatry. *Biol Psychiatry*. 2016; 79:150–152. [PubMed: 26723107]
21. Young LJ, Barrett CE. Can oxytocin treat autism? *Science*. 2015; 347:825–826. [PubMed: 25700501]
22. Walum H, Waldman ID, Young LJ. Statistical and Methodological Considerations for the Interpretation of Intranasal Oxytocin Studies. *Biol Psychiatry*. 2016; 79:251–257. [PubMed: 26210057]
23. Klimek R, Kordeusz Z. Cyclopeptide Hormones and Contraction of the Uterine Vessels. *Ginekol Pol*. 1963; 34:491–495. [PubMed: 14076229]
24. Hahn DW, Demarest KT, Ericson E, Himm RE, Capetola RJ, McGuire JL. Evaluation of 1-deamino-[D-Tyr(Oethyl)2, Thr4, Orn8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent. *Am J Obstet Gynecol*. 1987; 157:977–982. [PubMed: 3674173]
25. Muhlethaler M, Sawyer WH, Manning MM, Dreifuss JJ. Characterization of a uterine-type oxytocin receptor in the rat hippocampus. *Proc Natl Acad Sci U S A*. 1983; 80:6713–6717. [PubMed: 6314337]
26. Audigier S, Barberis C. Pharmacological characterization of two specific binding sites for neurohypophyseal hormones in hippocampal synaptic plasma membranes of the rat. *EMBO J*. 1985; 4:1407–1412. [PubMed: 2992930]
27. Carter CS, DeVries AC, Getz LL. Physiological substrates of mammalian monogamy: the prairie vole model. *Neurosci Biobehav Rev*. 1995; 19:303–314. [PubMed: 7630584]
28. de Wied D, Diamant M, Fodor M. Central nervous system effects of the neurohypophyseal hormones and related peptides. *Front Neuroendocrinol*. 1993; 14:251–302. [PubMed: 8258377]
29. Argiolas A, Collu M, D'Aquila P, Gessa GL, Melis MR, Serra G. Apomorphine stimulation of male copulatory behavior is prevented by the oxytocin antagonist d(CH2)5 Tyr(Me)-Orn8-vasotocin in rats. *Pharmacol Biochem Behav*. 1989; 33:81–83. [PubMed: 2780793]

30. Insel TR, Hulihan TJ. A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behav Neurosci.* 1995; 109:782–789. [PubMed: 7576222]
31. Sarnyai Z, Babarczy E, Krivan M, Szabo G, Kovacs GL, Barth T, Telegdy G. Selective attenuation of cocaine-induced stereotyped behaviour by oxytocin: putative role of basal forebrain target sites. *Neuropeptides.* 1991; 19:51–56. [PubMed: 1891073]
32. Pohl O, Homery MC, Lemaux F, Patat A, Chollet A. Pharmacokinetic interactions of OBE001 and betamethasone in healthy female volunteers. *J Clin Pharm Ther.* 2015; 40:328–332. [PubMed: 25899007]
33. Boccia ML, Goursaud AP, Bachevalier J, Anderson KD, Pedersen CA. Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. *Horm Behav.* 2007; 52:344–351. [PubMed: 17583705]
34. Smith AL, Freeman SM, Voll RJ, Young LJ, Goodman MM. Carbon-11 N-methyl alkylation of L-368,899 and in vivo PET imaging investigations for neural oxytocin receptors. *Bioorg Med Chem Lett.* 2013; 23:902–906. [PubMed: 23270988]
35. Striepens N, Kendrick KM, Hanking V, Landgraf R, Wuellner U, Maier W, Hurlmann R. Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Scientific Reports.* 2013; 3
36. Quintana DS, Alvares GA, Hickie IB, Guastella AJ. Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neurosci Biobehav Rev.* 2015; 49:182–192. [PubMed: 25526824]
37. Modi ME, Connor-Stroud F, Landgraf R, Young LJ, Parr LA. Aerosolized oxytocin increases cerebrospinal fluid oxytocin in rhesus macaques. *Psychoneuroendocrinology.* 2014; 45:49–57. [PubMed: 24845176]
38. Freeman SM, Samineni S, Allen PC, Stockinger D, Bales KL, Hwa GG, Roberts JA. Plasma and CSF oxytocin levels after intranasal and intravenous oxytocin in awake macaques. *Psychoneuroendocrinology.* 2016; 66:185–194. [PubMed: 26826355]
39. Preti A, Melis M, Siddi S, Vellante M, Doneddu G, Fadda R. Oxytocin and autism: a systematic review of randomized controlled trials. *J Child Adolesc Psychopharmacol.* 2014; 24:54–68. [PubMed: 24679173]
40. Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc Natl Acad Sci U S A.* 2010; 107:4389–4394. [PubMed: 20160081]
41. Koch SB, van Zuiden M, Nawijn L, Frijling JL, Veltman DJ, Olf M. Intranasal oxytocin as strategy for medication-enhanced psychotherapy of PTSD: salience processing and fear inhibition processes. *Psychoneuroendocrinology.* 2014; 40:242–256. [PubMed: 24485496]
42. Behnia B, Heinrichs M, Bergmann W, Jung S, Germann J, Schedlowski M, Hartmann U, Kruger TH. Differential effects of intranasal oxytocin on sexual experiences and partner interactions in couples. *Horm Behav.* 2014; 65:308–318. [PubMed: 24503174]
43. Colonnello V, Heinrichs M. Intranasal oxytocin enhances positive self-attribution in healthy men. *J Psychosom Res.* 2014; 77:415–419. [PubMed: 25439340]
44. Domes G, Kumbier E, Heinrichs M, Herpertz SC. Oxytocin promotes facial emotion recognition and amygdala reactivity in adults with asperger syndrome. *Neuropsychopharmacology.* 2014; 39:698–706. [PubMed: 24067301]
45. Leng G, Ludwig M. Intranasal Oxytocin: Myths and Delusions. *Biol Psychiatry.* 2016; 79:243–250. [PubMed: 26049207]
46. Neumann ID, Maloumy R, Beiderbeck DI, Lukas M, Landgraf R. Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology.* 2013; 38:1985–1993. [PubMed: 23579082]
47. Smith AL, Freeman SM, Stehouwer JS, Inoue K, Voll RJ, Young LJ, Goodman MM. Synthesis and evaluation of C-11, F-18 and I-125 small molecule radioligands for detecting oxytocin receptors. *Bioorg Med Chem.* 20:2721–2738.

48. Smith AL, Freeman SM, Voll RJ, Young LJ, Goodman MM. Investigation of an F-18 oxytocin receptor selective ligand via PET imaging. *Bioorg Med Chem Lett*. 2013; 23:5415–5420. [PubMed: 23978650]
49. Freeman SM, Inoue K, Smith AL, Goodman MM, Young LJ. The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (*Macaca mulatta*). *Psychoneuroendocrinology*. 2014; 45:128–141. [PubMed: 24845184]
50. Freeman SM, Young LJ. Comparative Perspectives on Oxytocin and Vasopressin Receptor Research in Rodents and Primates: Translational Implications. *J Neuroendocrinol*. 2016; 28
51. Dolen G, Darvishzadeh A, Huang KW, Malenka RC. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature*. 2013; 501:179–184. [PubMed: 24025838]
52. Buiter HJ, van Velden FH, Leysen JE, Fisher A, Windhorst AD, Lammertsma AA, Huisman MC. Reproducible Analysis of Rat Brain PET Studies Using an Additional [(18)F]NaF Scan and an MR-Based ROI Template. *Int J Mol Imaging*. 2012; 2012:580717. [PubMed: 23050141]
53. Smith AL, Freeman SM, Barnhart TE, Abbott DH, Ahlers EO, Kukis DL, Bales KL, Goodman MM, Young LJ. Initial investigation of three selective and potent small molecule oxytocin receptor PET ligands in New World monkeys. *Bioorg Med Chem Lett*. 2016; 26:3370–3375. [PubMed: 27209233]

ALS-I-41

 $hK_i = 16\text{nM}$

I-368,899

 $hK_i = 17\text{nM}$ **Figure 1.**

Structures of ALS-I-41 and I-386,899 and their affinity (K_i) for the human OXTR as determined by the Psychoactive Drug Screening Program at the University of North Carolina, Chapel Hill.

Figure 2.

Graphs indicating the amounts of ALS-I-41 found in the plasma (ng/ml) and the CSF (pg/ml) for low dose IN, High Dose IN, and IM direct injection. Note the y-axis is in ng/ml for plasma and pg/ml for CSF.

A.

B.

Figure 3.

Time-activity curves from the brain scan of a cynomolgus macaque showing A) the amount of SUV detected in the whole brain versus muscle tissue and B) regional brain SUV measured in the whole brain, brainstem, cerebellum, temporal lobe, frontal lobe, occipital lobe, parietal lobe, and the thalamus.

Figure 4. Images of [^{18}F]ALS-I-41 summed up over a 2 hour scan after injection into a cynomolgus macaque. The yellow arrow indicates the temporal lobe. The blue arrow indicates the frontal lobe. The red arrow indicates the cerebellum. The green arrow indicates the brainstem.

Figure 5.

In situ hybridization images of brain slices containing macaque cerebellar tissue generated from S-35 labelled probes. The slices are numbered to indicate their pairing. The red arrows indicate the signals obtained in the cerebellar region with the S-35 sense and antisense probes.

Figure 6.

A) Sum of minutes 9–26 post injection of 5 mCi of [^{18}F]ALS-I-41 into a cynomolgus macaque showing the relative equilibrium of activity throughout the entire brain. B) Sum of minutes 90–120 from the same injection showing the unequal distribution of activity throughout the same region of the brain at the end of the scan.

Figure 7.

Time-activity curves with standard deviations generated from $[^{18}\text{F}]\text{ALS-I-41}$ in rats from an IV bolus injection (n=3), 30 minute continuous IV infusion injection (n=2), and an IM injection (n=2).

Figure 8.

The last 60 minutes of a scan of $[^{18}\text{F}]$ ALS-I-41 after A) injection of 342 μCi via IM B) injection of 126 μCi via continuous IV infusion for 30 minutes and C) injection of 295 μCi via IV bolus injection. The blue arrows indicate the brain region.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Summary of ALS-I-41 administration and accumulation in plasma and CSF in male rhesus monkeys (average weight 11kg).

Route	Ave Dose (µg)	Max Dose (µg)	Min Dose (µg)	Ave Plasma Max (ng/ml)	Time Plasma Max (m)	Ave Max in CSF (pg/ml)	Time CSF Max (m)
IN	202	240	156	1.16	5	7.36	120
IN	7690	8000	7380	156	15	97.2	60
IM	6150	6150	6150	740	60	478	120

Table 2

Estimated concentrations of ALS-I-41 in macaque CSF assuming 10 ml total CSF volume and estimated target concentrations based on measured OT concentrations before and after aerosolized IN administration of 24 IU OT from Modi et al.

Route	Dose (mg)	Max [ALS-I-41] CSF (pM)	Time Max (m)	Estimated Effective Concentration (pM)	Estimated Blocking Concentration (pM)
IN	7.69	16	60	24	56
IM	6.15	85	120	24	56