Comment on “In Vivo [18 F]GE-179 Brain Signal Does Not Show NMDA-Specific Modulation with Drug Challenges in Rodents and Nonhuman Primates”

DOI:

[10.1021/acscchemneuro.8b00246](https://doi.org/10.1021/acscchemneuro.8b00246)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

McGinnity, C. J., Årstad, E., Beck, K., Brooks, D. J., Coles, J. P., Duncan, J. S., Galovic, M., Hinz, R., Hirani, E., Howes, O. D., Jones, P. A., Koepp, M. J., Luo, F., Riaño Barros, D. A., Singh, N., Trigg, W., & Hammers, A. (2018). Comment on “In Vivo [18 F]GE-179 Brain Signal Does Not Show NMDA-Specific Modulation with Drug Challenges in Rodents and Nonhuman Primates”. *ACS Chemical Neuroscience*.
<https://doi.org/10.1021/acscchemneuro.8b00246>

Published in:

ACS Chemical Neuroscience

Citing this paper

Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights

Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy

If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [<http://man.ac.uk/04Y6Bo>] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.

Comment on “*In Vivo* [¹⁸F]GE-179 Brain Signal Does Not Show NMDA-Specific Modulation with Drug Challenges in Rodents and Nonhuman Primates”

Authors

Colm J. McGinnity^{1, 2*}, Erik Årstad³, Katherine Beck⁴, David J. Brooks⁵, Jonathan P. Coles⁶, John S. Duncan⁷, Marian Galovic^{7, 8}, Rainer Hinz⁹, Ella Hirani¹⁰, Oliver D. Howes⁴, Paul A. Jones¹⁰, Matthias J. Koepp⁷, Feng Luo¹⁰, Daniela A. Riaño Barros¹¹, Nisha Singh^{1, 12}, William Trigg¹³, Alexander Hammers^{1, 2}.

Affiliations

¹School of Biomedical Engineering and Imaging Sciences, King's College London, UK

²King's College London & Guy's and St Thomas' PET Centre, St Thomas' Hospital, UK

³Organic Chemistry and Chemical Biology Section, Department of Chemistry, University College London, UK

⁴Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK

⁵Department of Nuclear Medicine, Aarhus University, Denmark, and Institute of Neuroscience, Newcastle University, UK

⁶Division of Anaesthesia, Department of Medicine, University of Cambridge, UK

⁷Department of Clinical and Experimental Epilepsy, University College London Institute of Neurology, UK, and Epilepsy Society, UK

⁸Department of Neurology, Kantonsspital St Gallen, Switzerland

⁹Wolfson Molecular Imaging Centre, University of Manchester, UK

- 24 ¹⁰GE Healthcare Ltd, UK
- 25 ¹¹South London and Maudsley NHS Foundation Trust, UK
- 26 ¹²Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College
- 27 London, UK
- 28 ¹³GE Healthcare Ltd, UK; current affiliation Adaptimmune Ltd, Abingdon, UK

Abstract

Schoenberger and colleagues (2018; ACS Chem. Neurosci. 9, 298-305) recently reported attempts to demonstrate specific binding of the positron emission tomography (PET) radiotracer, [^{18}F]GE-179, to NMDA receptors in both rats and Rhesus macaques. GE-179 did not work as expected in animal models; however, we disagree with the authors' conclusion that "the [^{18}F]GE-179 signal seems to be largely nonspecific".

It is extremely challenging to demonstrate specific binding for the use-dependent NMDA receptor intrachannel ligands such as [^{18}F]GE-179 in animals via traditional blocking, due to its low availability of target sites (B'_{max}). Schoenberger and colleagues anaesthetised rats and rhesus monkeys using isoflurane, which has an inhibitory effect on NMDA receptor function and thus would be expected to further reduce the B'_{max} .

The extent of glutamate release achieved in the provocation experiments is uncertain, as is whether a significant increase in NMDA receptor channel opening can be expected under anaesthesia.

Prior data suggest that the uptake of di-substituted arylguanidine-based ligands such as GE-179 can be reduced by phencyclidine binding site antagonists, if injection is performed *in the absence of ketamine and isoflurane anaesthesia*, e.g. with GE-179's antecedent, CNS 5161 (Biegon et al., 2007), and with GMOM (van der Doef et al., 2016). However, the extent of non-specific uptake remains uncertain.

Keywords

[^{11}C]CNS 5161, [^{18}F]GE-179, isoflurane, ketamine, NMDA, PET.

51 Graphic for Table of Contents

52
53

54 Introduction

55 Alterations in N-methyl D-aspartate (NMDA) receptor activation are implicated in the
56 pathophysiology of several neuropsychiatric disorders, including epilepsy, schizophrenia and
57 traumatic brain injury. Imaging NMDA receptor activation *in vivo* has proven challenging¹. [¹⁸F]GE-
58 179² is a candidate positron emission tomography (PET) radiotracer for this purpose that has shown
59 expected changes in both rat and human studies³⁻⁵. Schoenberger and colleagues recently reported
60 attempts to demonstrate specific binding of [¹⁸F]GE-179 to NMDA receptors in both rats and Rhesus
61 macaques⁶ in well-founded experiments simultaneously combining PET and magnetic resonance
62 imaging (MRI). Although the experiments conducted provide solid evidence that GE-179 does not
63 work as expected in animal models, we disagree with the authors' conclusion that "the [¹⁸F]GE-179
64 signal seems to be largely nonspecific", for the reasons outlined below.

65 The challenge of evaluating use-dependent PCP-site radiotracers

66 Unlike most other neuroreceptor radiotracers, [¹⁸F]GE-179 uptake is expected to reflect not only
67 receptor distribution but also receptor "state", i.e. it should exhibit "use-dependency". The
68 proportion of NMDA receptors that are in the open state at any one point of time in healthy rodents,
69 macaques and humans is unknown and the estimates of the probability of channel opening vary
70 considerably (e.g. 0.002⁶ – 0.3⁷). We believe that it is extremely challenging to demonstrate specific
71 binding for [¹⁸F]GE-179 in animals via traditional blocking as in ⁶, due to its low and inconstant
72 availability of target sites (B'_{max}).

73 Effects of anaesthesia on PCP-site availability

74 GE-179 (*N*-[2-chloro-5-(2-fluoroethylsulfanyl)phenyl]-*N'*-methyl-*N'*-(3-methylsulfanylphenyl)
75 guanidine)² is one of several putative di-substituted arylguanidine-based ligands with selectivity for
76 the intrachannel phencyclidine (PCP) binding site of the NMDA receptor. Other molecules in this
77 class that have been radiolabelled for imaging purposes include CNS 1261⁷, CNS 5161⁸, GMOM⁹ and

PK-209¹⁰ (Figure 1). The PCP binding site only becomes available when the receptor is in the “open” state, i.e. on simultaneous binding of both the agonist glutamate and a co-agonist such as glycine, accompanied by cell depolarisation.

Blocking studies of putative PCP-site NMDA-selective radiotracers are confounded by the use of general anaesthesia. In reference ⁶, the rats were anaesthetised via isoflurane inhalation, and the macaques were anaesthetised with ketamine and xylazine, with maintenance via isoflurane inhalation. The use of anaesthesia facilitates the acquisition of high-quality images. However, what is the effect of isoflurane, and the other anaesthetics used on B'_{max} ?

Isoflurane and similar volatile anaesthetics have complex mechanisms of action which include a well-described inhibitory effect on NMDA receptor function¹¹⁻²⁵, and which is possibly mediated in part via competitive antagonism at the glutamate²⁶ or glycine binding sites^{24, 26-28}. Such inhibition would be expected to reduce the already-low B'_{max} of PCP-site radiotracers such as [¹⁸F]GE-179. Demonstration of signal blockade in such circumstances would be extremely difficult.

Effects of methamphetamine on PCP site availability

In an attempt to increase B'_{max} via provocation of NMDA receptor channel opening, Schoenberger and colleagues⁶ injected methamphetamine two minutes prior to injection of [¹⁸F]GE-179 in rat studies and at 48 minutes p.i. in macaques studies, i.e. presumably administered after the induction of anaesthesia. Although *single*-dose methamphetamine may induce glutamate release²⁹⁻³⁰, there is some evidence that suggests the effect on glutamate release is negligible³¹⁻³². As the authors acknowledged, amphetamine and methamphetamine can actually directly inhibit the NMDA receptor³³⁻³⁵. It is not clear whether significant glutamate release was actually achieved, and whether and when a significant increase in NMDA receptor channel opening can be expected under the competing influences of anaesthesia and perhaps methamphetamine.

cortex-to-cerebellum uptake ratio from 1.45 to 1.20 (i.e. ~17%), approximately, at 90 minutes post-injection of [³H]CNS 5161³⁷. Whilst complete activation of the NMDA channel is unlikely with pharmacological manipulation at doses that do not elicit seizures, pre-treatment with NMDA 40mg/kg five minutes prior to injection of radiotracer increased the uptake ratio from 1.45 to 1.60 (i.e. ~10%), approximately, with larger increases (~31%) seen in the hippocampus. Approximately 17% blockade of signal has been seen in rats that were not sedated at the time of injection with [¹²³I]CNS-1261⁷.

Pre-treatment of a baboon with (cold) MK-801 (*after induction of anaesthesia with ketamine and maintenance with isoflurane*) did not significantly reduce [¹¹C]GMOM binding⁹, and similar to the results presented in reference ⁶, a slight increase was actually observed. Crucially, however, pre-treatment of *awake* rats with MK-801 (1 mg/kg intravenous) five minutes prior to injection produced a uniform decrease in binding of up to 28%⁹. The discrepant findings between awake rats and anaesthetised baboons are consistent with an anaesthesia-induced reduction of B'_{max} , *in vivo*. Pre-treatment of the awake rats with the co-agonist D-serine produced increases in binding of up to 24%, whereas the NR2B-selective antagonist Ro25-6981 produced decreases of up to 38%. Blockade of the binding of a [¹⁸F]PK-209, a [¹¹C]GMOM derivative, has also been seen with MK-801 pre-treatment in awake rats¹⁰, and in Rhesus macaques that were anaesthetised using agents other than ketamine and isoflurane³⁸.

Awake humans

A uniform decrease in [¹¹C]GMOM influx constant of approximately 66% was observed in six healthy, non-sedated human participants following early and prolonged administration of the low-affinity PCP-site antagonist, S-ketamine³⁹. The decrease in radioactivity concentration (kBq/ml), as opposed to influx constant, was not quantified but appeared to be modest (see “Data Analysis section below) – opposed by a slight (7%) increase in perfusion/extraction and accumulation in the non-specific compartment (V_{ND} ; 10%). A reduction in volume-of-distribution (V_T) of approximately 20% has also been observed with [¹²³I]CNS-1261⁴⁰⁻⁴¹.

Data Analysis

In the rat experiments⁶, Schoenberger et al inferred the absence of an effect from the failure to observe 'a meaningful change in the whole-brain TAC'. Whilst in some experiments the displacement is so clear simple assessment of the whole-brain time-activity curve will suffice⁴², a more rigorous quantitative analysis of the data is usually required to establish the presence of an effect. Drug competition can cause changes of the bioavailability of the PET tracer due to changes of the peripheral metabolism, alterations of the delivery, binding to peripheral sites, etc. Therefore model-based quantification of regional tracer binding in brain tissue is usually preferred over simpler methods when the expected effect sizes are in the order of a few per cent⁴³. The possibility cannot be excluded that the authors missed small but measureable effects in their *in vivo* rat experiments because they did not calculate quantitative measures of regional [¹⁸F]GE-179 binding.

Discussion

Taken together, these data suggest that the uptake/binding of di-substituted arylguanidine-based NMDA-selective radiotracers can be reduced by PCP site antagonists, if injection is performed *in the absence of ketamine and isoflurane anaesthesia*. We expect that this should be the case for [¹⁸F]GE-179, particularly since it has already been reported for its antecedent [¹¹C]CNS 5161³⁷.

Moreover, increased uptake/binding in non-sedated specimens has been demonstrated via direct provocation of channel opening with the agonist NMDA³⁷ and alternatively with the co-agonist D-serine⁹. Increased uptake/binding has also been demonstrated in conditions in which increased "endogenous" NMDA receptor channel opening is expected, such as deep brain stimulation⁴⁴, dyskinesias⁴⁵, epilepsy^{5, 44}, and cerebral ischaemia⁴⁶. There is good evidence, therefore, that these radiotracers specifically bind to the PCP site *in vivo*, and we suggest that the divergent findings in⁶ are explicable by the use of isoflurane and/or ketamine anaesthesia. A within-subject paired study design, in which [¹⁸F]GE-179 is administered prior to anaesthesia for one scan and subsequent to anaesthesia in the other, would allow this hypothesis to be tested. If confirmed and if it proves

valuable to perform pharmacological (or other) challenge before the induction of anaesthesia, this would advantage F-18 labelled agents such as [¹⁸F]GE-179 over those limited by the short radioactive half-life of C-11 (e.g. [¹¹C]GMOM).

The extent of blockade or alternatively the extent of enhancement that has been achieved thus far, in terms of change in radioactivity concentration, has been modest. The results presented in reference ³⁹ suggest that alterations in perfusion/extraction can confound the detection of blockade (and presumably enhancement). The modest alterations in signal might also have resulted in part from incomplete receptor blockade/enhancement; for example, Van der Doef and colleagues estimated that their ketamine dosing regimen (total 0.3 mg/kg over 135 minutes) resulted in an average occupancy of the PCP binding site of only approximately 19%³⁹. Hence, it is not immediately apparent that non-specific binding should be particularly marked for GE-179, given its lipophilicity ($\text{LogD}_{7.4} = 2.5 \pm 0.1$). However, the low volume of distribution observed for the second compartment (V_s) of kinetic models is consistent with low specific binding in healthy specimens.

In conclusion, we believe that the experiments described in reference ⁶, which contrast with those of several related studies^{9-10, 37, 39-41, 47-48}, do not adequately resolve the question of specific versus nonspecific binding for [¹⁸F]GE-179 and similar radiotracers. Evaluation of NMDA receptor-selective radiotracers is a challenging endeavour that will require continued experimental innovation. The data to date suggests that diarylguanidine-based PCP-site tracers are sensitive to channel opening in awake specimens, whereas the extent of non-specific uptake remains uncertain. [¹⁸F]GE-179 and [¹¹C]GMOM might still find use in clinical populations in which marked alterations in channel opening probability are expected.

Author Information

*Corresponding author:

Colm J. McGinnity (CJM)

School of Biomedical Engineering and Imaging Sciences

188 4th Floor Lambeth Wing
189 St Thomas' Hospital
190 Westminster Bridge Road
191 London, SE1 7EH
192 UK
193 Telephone: +44-(0)20 7188 9259
194 Fax: +44-(0)20 7188 3056
195 Email: colm.mcginney@kcl.ac.uk

196 Author Contributions

197 All authors contributed to preparation of the manuscript.

198 Funding Sources

199 AH&CJM, JPC, MG, MJK, and NS are currently supported by the Medical Research Council (UK;
200 MR/N013042/1; MR/L013215/1 and MR/K02308X/1; MR/L013215/1; MR/L013215/1; and
201 MR/K022733/1, respectively).

202 Conflicts of Interest

203 CJM, DARB, WT, DJB, JSD, MJK, and AH have conducted a study that used [¹⁸F]GE-179 and was
204 supported in part by GE Healthcare Ltd. EH, FL and JS are employees of GE Healthcare Ltd. EA and
205 WT were employees of GE Healthcare Ltd. CJM, JSD, and MJK have received fees from GE Healthcare
206 Ltd, but have never been employees of the organisation. JSD has also received fees from UCB
207 Pharma, Eisai, and GSK. The remaining authors do not declare any conflicts of interest.

208 References

- 209 1. Waterhouse, R. N. **(2003)** Imaging the PCP site of the NMDA ion channel. *Nucl. Med. Biol.* 30, 869-
210 878.
211 2. Robins, E. G., Zhao, Y., Khan, I., Wilson, A., Luthra, S. K., and Årstad, E. **(2010)** Synthesis and in vitro
212 evaluation of ¹⁸F-labelled S-fluoroalkyl diarylguanidines: Novel high-affinity NMDA receptor
213 antagonists for imaging with PET. *Bioorg. Med. Chem. Lett.* 20, 1749-1751.

3. Lopez-Picon, F., Snellman, A., Shatillo, O., Lehtiniemi, P., Gronroos, T. J., Marjamaki, P., Trigg, W., Jones, P. A., Solin, O., Pitkanen, A., and Haaparanta-Solin, M. **(2016)** Ex Vivo Tracing of NMDA and GABA-A Receptors in Rat Brain After Traumatic Brain Injury Using ^{18}F -GE-179 and ^{18}F -GE-194 Autoradiography. *J. Nucl. Med.* 57, 1442-7.
4. McGinnity, C. J., Hammers, A., Riano Barros, D. A., Luthra, S. K., Jones, P. A., Trigg, W., Micallef, C., Symms, M. R., Brooks, D. J., Koepp, M. J., and Duncan, J. S. **(2014)** Initial evaluation of ^{18}F -GE-179, a putative PET Tracer for activated N-methyl D-aspartate receptors. *J. Nucl. Med.* 55, 423-30.
5. McGinnity, C. J., Koepp, M. J., Hammers, A., Riano Barros, D. A., Pressler, R. M., Luthra, S., Jones, P. A., Trigg, W., Micallef, C., Symms, M. R., Brooks, D. J., and Duncan, J. S. **(2015)** NMDA receptor binding in focal epilepsies. *J. Neurol. Neurosurg. Psychiatry* 86, 1150-7.
6. Schoenberger, M., Schroeder, F. A., Placzek, M. S., Carter, R. L., Rosen, B. R., Hooker, J. M., and Sander, C. Y. **(2018)** In Vivo [^{18}F]GE-179 Brain Signal Does Not Show NMDA-Specific Modulation with Drug Challenges in Rodents and Nonhuman Primates. *ACS Chem. Neurosci.* 9, 298-305.
7. Owens, J., Tebbutt, A. A., McGregor, A. L., Kodama, K., Magar, S. S., Perlman, M. E., Robins, D. J., Durant, G. J., and McCulloch, J. **(2000)** Synthesis and binding characteristics of N-(1-naphthyl)-N'-(3-[^{125}I]-iodophenyl)-N'-methylguanidine ([^{125}I]-CNS 1261): a potential SPECT agent for imaging NMDA receptor activation. *Nucl. Med. Biol.* 27, 557-564.
8. Zhao, Y., Robins, E., Turton, D., Brady, F., Luthra, S. K., and Årstad, E. **(2006)** Synthesis and characterization of N-(2-chloro-5-methylthiophenyl)-N'-(3-methylthiophenyl)-N'-[^{11}C]methylguanidine [^{11}C]CNS 5161, a candidate PET tracer for functional imaging of NMDA receptors. *J. Labelled Comp. Radiopharm.* 49, 163-170.
9. Waterhouse, R. N., Slifstein, M., Dumont, F., Zhao, J., Chang, R. C., Sudo, Y., Sultana, A., Balter, A., and Laruelle, M. **(2004)** In vivo evaluation of [^{11}C]N-(2-chloro-5-thiomethylphenyl)-N'-(3-methoxyphenyl)-N'-methylguanidine ([^{11}C]GMOM) as a potential PET radiotracer for the PCP/NMDA receptor. *Nucl. Med. Biol.* 31, 939-948.
10. Klein, P. J., Schuit, R. C., Metaxas, A., Christiaans, J. A. M., Kooijman, E., Lammertsma, A. A., van Berckel, B. N. M., and Windhorst, A. D. **(2017)** Synthesis, radiolabeling and preclinical evaluation of a [^{11}C]GMOM derivative as PET radiotracer for the ion channel of the N-methyl-D-aspartate receptor. *Nucl. Med. Biol.* 51, 25-32.
11. Yamakura, T., and Harris, R. A. **(2000)** Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol. *Anesthesiology* 93, 1095-101.
12. Yang, J., and Zorumski, C. F. **(1991)** Effects of isoflurane on N-methyl-D-aspartate gated ion channels in cultured rat hippocampal neurons. *Ann. N. Y. Acad. Sci.* 625, 287-9.
13. Solt, K., Eger, E. I., 2nd, and Raines, D. E. **(2006)** Differential modulation of human N-methyl-D-aspartate receptors by structurally diverse general anesthetics. *Anesth. Analg.* 102, 1407-11.
14. Hollmann, M. W., Liu, H. T., Hoenemann, C. W., Liu, W. H., and Durieux, M. E. **(2001)** Modulation of NMDA receptor function by ketamine and magnesium. Part II: interactions with volatile anesthetics. *Anesth. Analg.* 92, 1182-91.
15. Ogata, J., Shiraishi, M., Namba, T., Smothers, C. T., Woodward, J. J., and Harris, R. A. **(2006)** Effects of anesthetics on mutant N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *J. Pharmacol. Exp. Ther.* 318, 434-43.
16. Eger, E. I., 2nd, Liao, M., Laster, M. J., Won, A., Popovich, J., Raines, D. E., Solt, K., Dutton, R. C., Cobos, F. V., 2nd, and Sonner, J. M. **(2006)** Contrasting roles of the N-methyl-D-aspartate receptor in the production of immobilization by conventional and aromatic anesthetics. *Anesth. Analg.* 102, 1397-406.
17. Ranft, A., Kurz, J., Deuringer, M., Haseneder, R., Dodt, H. U., Zieglansberger, W., Kochs, E., Eder, M., and Hapfelmeier, G. **(2004)** Isoflurane modulates glutamatergic and GABAergic neurotransmission in the amygdala. *Eur. J. Neurosci.* 20, 1276-80.
18. Ming, Z., Griffith, B. L., Breese, G. R., Mueller, R. A., and Criswell, H. E. **(2002)** Changes in the effect of isoflurane on N-methyl-D-aspartic acid-gated currents in cultured cerebral cortical neurons with time in culture: evidence for subunit specificity. *Anesthesiology* 97, 856-67.

19. Berg-Johnsen, J., and Langmoen, I. A. **(1992)** The effect of isoflurane on excitatory synaptic transmission in the rat hippocampus. *Acta Anaesthesiol. Scand.* 36, 350-5.
20. Bickler, P. E., Buck, L. T., and Hansen, B. M. **(1994)** Effects of isoflurane and hypothermia on glutamate receptor-mediated calcium influx in brain slices. *Anesthesiology* 81, 1461-9.
21. Zuo, Z., De Vente, J., and Johns, R. A. **(1996)** Halothane and isoflurane dose-dependently inhibit the cyclic GMP increase caused by N-methyl-D-aspartate in rat cerebellum: novel localization and quantitation by *in vitro* autoradiography. *Neuroscience* 74, 1069-75.
22. Nishikawa, K., and MacIver, M. B. **(2000)** Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated responses. *Anesthesiology* 92, 228-36.
23. Carla, V., and Moroni, F. **(1992)** General anaesthetics inhibit the responses induced by glutamate receptor agonists in the mouse cortex. *Neurosci. Lett.* 146, 21-4.
24. Martin, D. C., Abraham, J. E., Plagenhoef, M., and Aronstam, R. S. **(1991)** Volatile anesthetics and NMDA receptors. Enflurane inhibition of glutamate-stimulated [³H]MK-801 binding and reversal by glycine. *Neurosci. Lett.* 132, 73-6.
25. Harada, H., Kelly, P. J., Cole, D. J., Drummond, J. C., and Patel, P. M. **(1999)** Isoflurane reduces N-methyl-D-aspartate toxicity *in vivo* in the rat cerebral cortex. *Anesth. Analg.* 89, 1442-7.
26. Martin, D. C., Plagenhoef, M., Abraham, J., Dennison, R. L., and Aronstam, R. S. **(1995)** Volatile anesthetics and glutamate activation of N-methyl-D-aspartate receptors. *Biochem. Pharmacol.* 49, 809-17.
27. Dickinson, R., Peterson, B. K., Banks, P., Simillis, C., Martin, J. C., Valenzuela, C. A., Maze, M., and Franks, N. P. **(2007)** Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor by the anesthetics xenon and isoflurane: evidence from molecular modeling and electrophysiology. *Anesthesiology* 107, 756-67.
28. Ishizaki, K., Yoshida, N., Yoon, D. M., Yoon, M. H., Sudoh, M., and Fujita, T. **(1996)** Intrathecally administered NMDA receptor antagonists reduce the MAC of isoflurane in rats. *Can. J. Anaesth.* 43, 724-30.
29. Stephans, S. E., and Yamamoto, B. K. **(1994)** Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. *Synapse* 17, 203-9.
30. Ruda-Kucerova, J., Amchova, P., Havlickova, T., Jerabek, P., Babinska, Z., Kacer, P., Syslova, K., Sulcova, A., and Sustkova-Fiserova, M. **(2015)** Reward related neurotransmitter changes in a model of depression: An *in vivo* microdialysis study. *World J. Biol. Psychiatry* 16, 521-35.
31. Stephans, S. E., and Yamamoto, B. Y. **(1995)** Effect of repeated methamphetamine administrations on dopamine and glutamate efflux in rat prefrontal cortex. *Brain Res.* 700, 99-106.
32. Shoblock, J. R., Sullivan, E. B., Maisonneuve, I. M., and Glick, S. D. **(2003)** Neurochemical and behavioral differences between d-methamphetamine and d-amphetamine in rats. *Psychopharmacology (Berl.)* 165, 359-69.
33. Yeh, G. C., Chen, J. C., Tsai, H. C., Wu, H. H., Lin, C. Y., Hsu, P. C., and Peng, Y. C. **(2002)** Amphetamine inhibits the N-methyl-D-aspartate receptor-mediated responses by directly interacting with the receptor/channel complex. *J Pharmacol. Exp. Ther.* 300, 1008-16.
34. Lee, K. W., Kim, H. C., Lee, S. Y., and Jang, C. G. **(2011)** Methamphetamine-sensitized mice are accompanied by memory impairment and reduction of N-methyl-d-aspartate receptor ligand binding in the prefrontal cortex and hippocampus. *Neuroscience* 178, 101-7.
35. Smith, K. J., Self, R. L., Butler, T. R., Mullins, M. M., Ghayoumi, L., Holley, R. C., Littleton, J. M., and Prendergast, M. A. **(2007)** Methamphetamine exposure antagonizes N-methyl-D-aspartate receptor-mediated neurotoxicity in organotypic hippocampal slice cultures. *Brain Res.* 1157, 74-80.
36. Hu, L. Y., Guo, J., Magar, S. S., Fischer, J. B., Burke-Howie, K. J., and Durant, G. J. **(1997)** Synthesis and pharmacological evaluation of N-(2,5-disubstituted phenyl)-N'-(3-substituted phenyl)-N'-methylguanidines as N-methyl-D-aspartate receptor ion-channel blockers. *J. Med. Chem.* 40, 4281-9.

37. Biegon, A., Gibbs, A., Alvarado, M., Ono, M., and Taylor, S. **(2007)** *In vitro* and *in vivo* characterization of [³H]CNS-5161--a use-dependent ligand for the N-methyl-D-aspartate receptor in rat brain. *Synapse* 61, 577-86.
38. Golla, S. S., Klein, P. J., Bakker, J., Schuit, R. C., Christiaans, J. A., van Geest, L., Kooijman, E. J., Oropeza-Seguias, G. M., Langermans, J. A., Leysen, J. E., Boellaard, R., Windhorst, A. D., van Berckel, B. N., and Metaxas, A. **(2015)** Preclinical evaluation of [¹⁸F]PK-209, a new PET ligand for imaging the ion-channel site of NMDA receptors. *Nucl. Med. Biol.* 42, 205-12.
39. van der Doef, T. F., Golla, S. S., Klein, P. J., Oropeza-Seguias, G. M., Schuit, R. C., Metaxas, A., Jobse, E., Schwarte, L. A., Windhorst, A. D., Lammertsma, A. A., van Berckel, B. N., and Boellaard, R. **(2016)** Quantification of the novel N-methyl-d-aspartate receptor ligand [¹¹C]GMOM in man. *J. Cereb. Blood Flow Metab.* 36, 1111-21.
40. Stone, J. M., Erlandsson, K., Arstad, E., Bressan, R. A., Squassante, L., Teneggi, V., Ell, P. J., and Pilowsky, L. S. **(2006)** Ketamine displaces the novel NMDA receptor SPET probe [¹²³I]CNS-1261 in humans in vivo. *Nucl. Med. Biol.* 33, 239-43.
41. Stone, J. M., Erlandsson, K., Arstad, E., Squassante, L., Teneggi, V., Bressan, R. A., Krystal, J. H., Ell, P. J., and Pilowsky, L. S. **(2008)** Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [¹²³I]CNS-1261 SPET study. *Psychopharmacology (Berl.)* 197, 401-8.
42. Malizia, A. L., Melichar, J. M., Brown, D. J., Gunn, R. N., Reynolds, A., Jones, T., and Nutt, D. J. **(1997)** Demonstration of clomipramine and venlafaxine occupation at serotonin reuptake sites in man *in vivo*. *J. Psychopharmacol.* 11, 279-281.
43. Lammertsma, A. A. **(2017)** Forward to the Past: The Case for Quantitative PET Imaging. *J. Nucl. Med.* 58, 1019-1024.
44. Vibholm, A., Christensen, J., Beniczky, S., Landau, A., Dietz, M., Moller, A., Christian Hedemann Sorensen, J., and Brooks, D. **(2017)** IN-VIVO IMAGING OF ACTIVATED NMDA RECEPTOR ION CHANNELS WITH THE RADIOLIGAND ¹⁸F-G179. *Epilepsia* 58 (Suppl. 5), S22.
45. Ahmed, I., Bose, S. K., Pavese, N., Ramlackhansingh, A., Turkheimer, F., Hotton, G., Hammers, A., and Brooks, D. J. **(2011)** Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. *Brain* 134, 979-86.
46. Zhou, W., Bao, W., Jiang, D., Kong, Y., Hua, F., Lu, X., and Guan, Y. **(2018)** [¹⁸F]-GE-179 positron emission tomography (PET) tracer for N-methyl-D-aspartate receptors: One-pot synthesis and preliminary micro-PET study in a rat model of MCAO. *Nucl. Med. Biol.* 61, 45-55.
47. Klein, P. J., Christiaans, J. A. M., Metaxas, A., Schuit, R. C., Lammertsma, A. A., van Berckel, B. N. M., and Windhorst, A. D. **(2015)** Synthesis, structure activity relationship, radiolabeling and preclinical evaluation of high affinity ligands for the ion channel of the N-methyl-d-aspartate receptor as potential imaging probes for positron emission tomography. *Bioorg. Med. Chem.* 23, 1189-1206.
48. Knol, R. J., de Bruin, K., van Eck-Smit, B. L., Pimlott, S., Wyper, D. J., and Booij, J. **(2009)** In vivo [¹²³I]CNS-1261 binding to D-serine-activated and MK801-blocked NMDA receptors: A storage phosphor imaging study in rats. *Synapse* 63, 557-64.