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- (71) **Applicant:** NOVARTIS AG [CH/CH]; Lichtstrasse 35, 4056 Basel (CH).
- (72) **Inventors:** BILL, Anke; Novartis Institutes for Biomedical Research Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). CAMARGO, Luiz Miguel Quinn; 6 Shirley Road, Wellesley, Massachusetts 02482 (US). DIGAN, Mary Ellen; Novartis Institutes of Biomed-

ical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). GUO, Hongqiu; Novartis Institutes of Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). RAO, Hao-Ran; 101 Magoun Avenue, Medford, Massachusetts 02155 (US).

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- (54) **Title:** A HIGH-THROUGHPUT ASSAY METHOD FOR IDENTIFYING ALLOSTERIC NMDA RECEPTOR MODULATORS

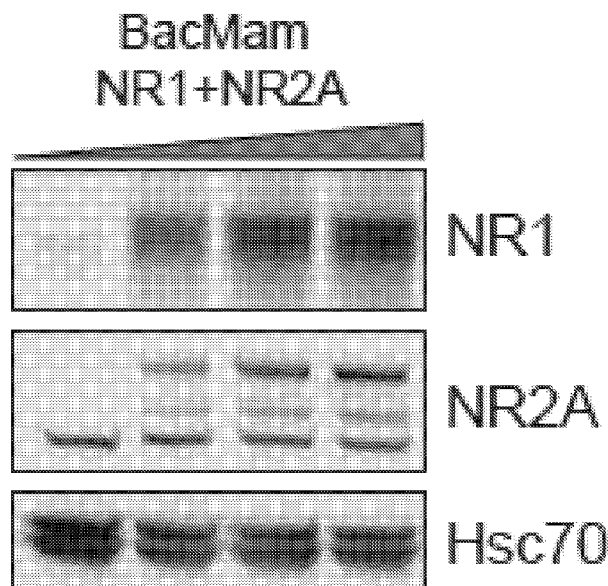


FIG. 1

- (57) **Abstract:** A novel, high-throughput assay and methods to study N-methyl-D-aspartate receptors (NMDARs). The method allows for rapid comparisons of different subunits, the ability to measure effects of agonists for both the glycine binding site and the glutamate binding site. The methods leverage the use of weak glycine and glutamate sites binding antagonists rather than to ketamine or MK-801 to mitigate against cellular toxicity and to retain the ligand binding site in a ligand-free state. The method can use baculovirus vectors to introduce titratable amounts of different receptor subunits. The assay replicates the pharmacology of NMDARs in response to known antagonists and allosteric modulators, as well as sensitivity to magnesium.



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A HIGH-THROUGHPUT ASSAY METHOD FOR IDENTIFYING ALLOSTERIC NMDA RECEPTOR MODULATORS

TECHNICAL FIELD OF THE INVENTION

5 **[001]** The invention relates generally to *in vitro* processes that test organic compounds for binding to a cell membrane bound receptor.

BACKGROUND OF THE INVENTION

10 **[002]** N-methyl-D-aspartate-receptors ("NMDA receptors" or "NMDARs") are ionotropic glutamate receptors that require binding of two ligands, *i.e.*, glutamate and co-factors glycine or D-serine for activity. NMDARs are complex, being composed of different subtypes with distinctive properties. See, Paoletti *et al.*, *Nature Rev. Neurosci.* 14: 383–400 (2013).

15 **[003]** Most marketed drugs that target NMDARs are antagonists that block the pathological effects of having an overly activated receptor. Enhancing the activity of NMDARs can result in excitotoxicity and unwanted adverse events. Chen *et al.*, *J. Neurochem.* 97(6): 1611-26 (2006). For example, the NMDA channel blocker memantine is administered for the treatment of Alzheimer's disease. The proposed mechanism is that memantine blocks A β 42 oligomers, the toxic agents in Alzheimer's disease, from
20 over-exciting NMDARs. Cacabelos *et al.*, *Int. J. Geriatr. Psychiatry* 14: 3–47 (1999).

25 **[004]** Schizophrenia (SZ) is a chronic, severe and disabling neuropsychiatric disease with an estimated prevalence of 1% in the general population. Negative and cognitive symptoms represent a great unmet medical need in SZ, since the impact of antipsychotics (current standard of care) is limited to the mitigation of positive symptoms, effective only in 60-70% of schizophrenia patients and is further limited by a number of severe adverse effects. Experimental evidence supports an involvement of NMDAR in the etiology and pathophysiology of schizophrenia. Normalization of NMDAR activity in the brain of patients suffering from schizophrenia is predicted to mitigate symptoms and to address the unmet medical need in this disease. Coyle J.T. *Schizophrenia Bulletin*.
30 38(5), 920–926 (2012).

35 **[005]** Several approaches have previously been used to study NMDARs in cell-based systems. Two approaches are calcium influx measurements and electrophysiological measurements. Paoletti *et al.*, *Nature Rev. Neurosci.* 14: 383–400 (2013). However, studying different NMDARs in cell-based systems is challenging. The limitations of currently available models include: (a) the high expression levels of functional NR2 subunit are cytotoxic, leading to cell death; (b) recombinant gene expression leads to a random ratio of subunits; (c) the need to use ketamine or similar strong inhibitors for protection against cytotoxicity leads to other disadvantages; and (d) cells are cultured in

presence of ketamine to protect from excitotoxicity are not sensitive to glycine/D-serine and have limited sensitivity to glutamine in plate based assay; and (e) high levels of glycine are both present in cell media and secreted by cultured cells upon their death.

[006] One *in vitro* approach to reducing toxicity has been to engineer cells expressing only one subunit constitutively, while the other subunit is expressed under the control of an inducible promotor prior to the experiment. Even in such systems, the presence of functional NMDARs is toxic, requiring that the cell cultures be maintained in the presence of potent channel blockers such as ketamine or dizocilpine (MK-801). Unfortunately, NMDAR channel blocker compounds are difficult to wash off the cells being tested, are themselves toxic to the cells and may confound measurements of calcium signaling in a high-throughput fashion. Furthermore, accelerated by the high amount of cell death, endogenous levels of glycine and glutamate are released by the cells in culture, which then occupy the ligand binding sites and maintain the NMDAR in an activated or desensitized state.

[007] Bettini *et al.*, *J. Pharmacol. Exp. Ther.* 335(3): 636–644 (2010) described an NMDAR fluorescence assay, using human osteosarcoma (U-2 OS) cells transiently transduced with NR1/2A NMDAR subunit, with detection on a fluorometric imaging plate reader (FLIPR). Bettini's assay used ketamine in the culture medium and the cells were washed to remove ketamine. Under Bettini's conditions NMDAR showed significant constitutive activity and little sensitivity to ligand stimulation.

[008] Feuerbach *et al.*, *Eur. J. Pharmacol.* 637: 46–54 (2010) described an assay that combined a muristerone-inducible expression system for the N1 subunit with constitutive expression of NMDA-R2A, 2B, 2C and 2D in different cell clones. Feuerbach's assay also used ketamine in the culture medium and the cells were washed to remove ketamine. Feuerbach's assay also only showed sensitivity to glutamate, not D-serine/glycine.

[009] Hansen *et al.*, *Combinatorial Chemistry & High Throughput Screening* 11: 304–315 (2008) described an NMDAR fluorescence assay with detection on a FLIPR. The authors established cell lines with stable expression of NMDAR subtypes NR1/2A, NR1/2B, NR1/2C, or NR1/2D in baby hamster kidney BHK-21 cells. Their assay did not use ketamine in the culture medium. Rather, the cell lines were cultured in the presence of both a competitive glycine binding site antagonist (7-CKA) and a competitive glutamate binding site antagonist (AP5), to prevent NMDAR-mediated cell death. Hansen's assay was not sensitive to glycine.

[010] Hansen *et al.*, *J. Pharmacol. Exp. Ther.* 333: 650–662 (2010) described a BHK-21 cell line stably expressing NR1/2D, where the expression of NR1 was under the control of a Tet-inducible promoter. The authors selected a cell line that displayed a

robust response to glutamate and glycine for use in their assay. Their assay did not use ketamine in the culture medium. Rather, the cell line was cultured in the presence of both 7-CKA and AP5, to prevent NMDAR-mediated cell death. Their assay also used the cell-permeable calcium indicator Fluo-4 NW (a fluorescence dye) to measure calcium flux
5 and contained 7-CKA to prevent cytotoxicity during the dye loading and during the assay. The NMDAR antagonists were displaced by addition of a high concentration of glycine (1 mM) and glutamate (100 μ M). Hansen's assay was sensitive to D-serine/glycine only in the presence of the inhibitor.

[011] Published U.S. patent application US2012028977A1 (Traynelis *et al.*) "Subunit
10 selective NMDA receptor potentiators" for the treatment of neurological conditions) discloses a cell-based assay to identify modulators that are selective for NR2C- or NR2D-containing receptors (NR1/NR2C or NR1/NR2D, but does not explicitly exclude the use of channel blockers.

[012] Published PCT patent publication WO 2015/052226 (Genentech) describes an
15 assay that uses a stable cell line with inducible expression of NMDAR. Because the stable/induced expression of NMDAR was toxic for the cells, 500 μ M ketamine was added to the cell cultures to protect against excitotoxicity. During the assay itself, 10 μ M ketamine was present.

[013] There remains a need in the neurological and biochemical arts for a rapid, flexible
20 and titratable *in vitro* NMDAR assay that does not use ketamine or similar channel blockers and that shows sensitivity to D-serine/glycine. Such an assay should be sensitive to glycine even without the presence of a blocker or other inhibitor. Such an assay should be useful as an unbiased assay for new NMDAR-binding compounds.

SUMMARY OF THE INVENTION

- [014]** The invention provides a method for identifying a modulator of N-methyl-D-aspartate receptor (NMDAR) function. In a first embodiment, the method includes the steps of (a) transiently expressing an NMDAR protein or protein subunit on the surface of a mammalian cell; (b) culturing the mammalian cell in a culture medium, where the culture medium (i) contains an NMDAR ligand binding site antagonist; and (ii) does not contain an NMDAR channel blocker; replacing (i) the culture medium that contains the NMDAR ligand binding site antagonist and does not contain an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker; (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell; (d) while retaining the mammalian cell, (e) measuring the calcium response by the mammalian cell; (f) contacting the mammalian cell with a test agent; (g) measuring the calcium response by the mammalian cell to the test agent; (h) contacting the mammalian cell with an NMDAR ligand site binder; and (i) measuring the calcium response by the mammalian cell. A change in calcium response by the mammalian cell identifies the test agent as a modulator of NMDAR function. In a second embodiment, the buffer or culture medium does not contain an NMDAR channel blocker or any other NMDAR inhibitor other than the test agent, when the test agent is an antagonist.
- [015]** In a third embodiment, transiently expressing NMDAR protein or protein subunit on the surface of the mammalian cell is accomplished by a transient transfection of the mammalian cell with a vector containing an NMDAR gene, and subsequent expression of an NMDAR gene. In a fourth embodiment, the vector is a vector containing one or multiple NMDAR genes under the control of a constitutively active mammalian promoter leading to transient expression of NMDAR. In a fifth embodiment, the vector is a baculovirus vector containing an NMDAR gene, such as a BacMam vector containing an NMDAR gene, such as a human NMDAR. In a sixth embodiment, the transiently expressing NMDAR protein or protein subunit on the surface of the mammalian cell is accomplished by a stable transfection or a lentiviral infection of the mammalian cell with one or more vectors comprising one or more NMDAR genes, where one or more of the NMDAR genes can be inducible, such as by doxycycline.
- [016]** In a seventh embodiment, the recombinant baculovirus vector containing an NMDAR gene contains one or more nucleotide sequences of a human NMDAR NR1 subunit and/or NR2 subunit together with additional sequences capable of directing the synthesis of the human NMDAR subunit in cultures of transiently transfected mammalian cells.

[017] In an eighth embodiment, the transiently expressed NMDAR is selected from the group consisting of NR1/2A and NR1/2B and NR1/2D. In a ninth embodiment, the NMDAR subunit is selected from the group consisting of NR1, NR2A, NR2B, and NR2D (mouse or human sequence variants).

5 **[018]** In a tenth embodiment, the transiently expressed NMDAR is a human NMDAR or a mouse NMDAR. In an eleventh embodiment, the mammalian cell is a human cell, such as a HEK293 cell or a human induced pluripotent stem cell (iPSC).

[019] In a twelfth embodiment, the cells in the assay are infected with BacMam virus encoding NMDAR genes. In a thirteenth embodiment, the cells in the assay are
10 protected from NMDAR-mediated excitotoxicity by the addition of NMDAR inhibitor into the media during the culture of the cells. In a fourteenth embodiment, the NMDAR ligand binding site antagonist binds to the glycine binding site of the NMDAR. In a fifteenth embodiment, the NMDAR ligand binding site antagonist binds to the glutamate binding site of the NMDAR. In a sixteenth embodiment, the NMDAR ligand binding site
15 antagonist used in the culturing step of the invention is MDL105, 519 or CGP070667. As demonstrated herein, the NMDAR channel blocker not used in the method of the invention can be, for example, ketamine, memantine or MK801.

[020] In a seventeenth embodiment, manipulating the mammalian cell so that it responds in a measurable way to changes in calcium concentration in the mammalian
20 cell is accomplished by loading the mammalian cell with a cell-permeable calcium indicator, such as a fluorescent calcium indicator. In an eighteenth embodiment, the fluorescent calcium indicator is calcium 6 dye.

[021] In an nineteenth embodiment, manipulating the mammalian cell so that it is responds in a measurable way to changes in calcium concentration in the mammalian
25 cell is accomplished by stably expressing a calcium sensor protein in the mammalian cell. The calcium sensor protein is stably expressed in the mammalian cell by expression from a lentivirus vector comprising a calcium sensor protein gene. In a twentieth embodiment, the calcium sensor protein is CGaMP6; in a twenty-first embodiment, CGaMP6 with two aspartic acid (D) -> cysteine (C) point mutations (dCys-GCaMP6)
30 (SEQ ID NO: 1); and in a twenty-second embodiment, CGaMP3 (SEQ ID NO: 2).

[022] In a twenty-third embodiment, the measuring of the calcium response by the mammalian cell is in a high-throughput screen. A high-throughput, calcium flux assay of the invention has been developed as a screening assay. The assay shows sensitivity to both endogenous NMDAR co-agonists, enabling measurements of NMDAR activity in the
35 presence of limiting amounts of either ligand. Importantly the readouts obtained from this assay exhibit expected NMDAR pharmacology.

[023] In a twenty-fourth embodiment, the NMDAR ligand site binder binds to the NMDAR glycine binding site. In a twenty-fifth embodiment, the NMDAR ligand site binder binds to the NMDAR glutamate binding site. In a twenty-sixth embodiment, one or more NMDAR ligand site binder is selected from the group consisting of glycine, L-glutamate, CGP070667, AAM077, 7-CTKA, MDL105,519, L701,324, CGP039653 and CPP.

[024] In a twenty-seventh embodiment, the modulator of NMDAR function is an NMDAR positive allosteric modulator, as detected by an increase in calcium response. In a twenty-eighth embodiment, the modulator of NMDAR function is an NMDAR negative inhibitor, as detected by a decrease in calcium response. In a twenty-ninth embodiment, the modulator of NMDAR function can bind to the NMDAR glycine binding site or to the NMDAR glutamate binding site. In thirtieth embodiment, the modulator can bind to any other site in NMDAR (by an allosteric mechanism).

[025] The invention also provides a mammalian cell having a high level of an NMDAR protein on the cell surface. In a thirty-first embodiment, the mammalian cell comprises a vector containing an expressible NMDAR gene, where (i) the vector is a baculovirus vector; or (ii) the vector is integrated in the mammalian cell genome, and measurable by resistance to a selection marker encoded by the vector; or (iii) the vector does not contain a selection marker and is only transiently expressed; the expressible NMDAR gene is expressed in the mammalian cell; the expressed NMDAR protein or protein subunit thereof is present on the cell surface; such that the mammalian cell is viable. The cell viability is measured by methods known in the art to show viability, such as – in a thirty-second embodiment - by measuring the amount of adenosine triphosphate (ATP) present, which indicates the presence of metabolically active cells.

[026] In a thirty-third embodiment, the transiently expressing NMDAR protein or protein subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector and expression of an NMDAR gene on the vector. In a thirty-fourth embodiment, the vector is a baculovirus vector containing an NMDAR gene, such as by the infection or transduction of a BacMam vector.

[027] In a thirty-fifth embodiment, the NMDAR is a human NMDAR. In a thirty-sixth embodiment, the NMDAR is a mouse NMDAR.

[028] The invention further provides a method for producing a viable mammalian cell having NMDAR on its surface. In a thirty-seventh embodiment, the method includes the steps of: (a) transiently transfecting the mammalian cell with a vector selected from (i) a baculovirus vector containing one or more expressible NMDAR genes, or a plasmid coding for NMDAR; and (b) culturing the mammalian cell, where the culture medium (i) contains an NMDAR ligand binding site antagonist; and (ii) does not contain an NMDAR channel blocker.

[029] In a thirty-eighth embodiment, transiently expressing NMDAR protein or subunit on the surface of the mammalian cell is accomplished by a transient transfection of the mammalian cell with a vector and expression of an NMDAR gene on the vector. In a thirty-ninth embodiment, the vector is a baculovirus vector containing an NMDAR gene, such as a BacMam vector.

[030] In a fortieth embodiment, the mammalian cell in the assay is a human cell, such as, in a forty-first embodiment, HEK293, or in a forty-second embodiment, a human iPSC.

[031] The invention provides a method for facilitating the sensitivity of NMDAR to ligands of the NMDAR glycine site or to the NMDAR glutamate site. In a forty-third embodiment, the method includes the steps of (a) transiently expressing NMDAR protein or protein subunits thereof on the surface of a mammalian cell; (b) culturing the mammalian cell in a culture medium, where the culture medium contains an NMDAR ligand binding site antagonist; but does not contain an NMDAR channel blocker; (c) then, while retaining the mammalian cell, replacing (i) the culture medium that contains the NMDAR ligand binding antagonist and does not contain an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker; (d) contacting the mammalian cell with a test agent; (e) contacting the mammalian cell with an NMDAR ligand site binder; and (f) determining whether the test agent binds to the glycine binding site or the glutamate binding site.

[032] In a forty-fourth embodiment, transiently expressing NMDAR protein or subunit on the surface of the mammalian cell is accomplished by a transient transfection of the mammalian cell with a vector and expression of an NMDAR gene on the vector. In a forty-fifth embodiment, the vector can be a baculovirus vector containing an NMDAR gene, such as a BacMam vector.

[033] In a forty-sixth embodiment, the method further includes, after the culturing step, manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell. In a forty-seventh embodiment, the method further includes, both after the contacting step (d) and after the contacting step (f), measuring the calcium response by the mammalian cell.

[034] The invention provides a method for dissecting the mechanism of action of NMDAR inhibitors. In a forty-eighth embodiment, the method includes the steps of (a) transiently expressing NMDAR protein or protein subunits on the surface of a mammalian cell; (b) culturing the mammalian cell in a culture medium that contains an NMDAR ligand binding antagonist, but does not contain an NMDAR channel blocker; then (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell; (d) while retaining the mammalian cell,

replacing the culture medium that contains the NMDAR ligand binding antagonist and does not contain an NMDAR channel blocker with a buffer or culture medium that does not contain an NMDAR channel blocker; (e) measuring the calcium response by the mammalian cell; (f) contacting the mammalian cell with a test agent; (g) measuring the calcium response by the mammalian cell to the test agent; (h) contacting the mammalian cell with an NMDAR ligand site binder; (i) again measuring the calcium response by the mammalian cell; and (j) determining whether the test agent binds to the glycine binding site or the glutamate binding site.

[035] In a forty-ninth embodiment, transiently expressing NMDAR protein or protein subunit on the surface of the mammalian cell is accomplished by a transient transfection of the mammalian cell with a vector and expression of an NMDAR gene on the vector. In a fiftieth embodiment, the vector can be a baculovirus vector containing an NMDAR gene, such as a BacMam vector containing an NMDAR gene.

[036] The invention provides a method for detecting calcium levels in a eukaryotic cell. In a fifty-first embodiment, the method includes the steps of: (a) transfecting the eukaryotic cell with a polynucleotide that contains an expressible gene encoding a calcium sensor (GECS)-based GCaMP-protein; (b) expressing the gene for a genetically encoded calcium sensor to create a eukaryotic cell stably expressing GCaMP protein; (c) contacting the eukaryotic cell with a test agent; and (d) measuring the calcium response by the eukaryotic cell to the test agent.

[037] In a fifty-second embodiment, the polynucleotide is a lentivirus.

[038] In a fifty-third embodiment, the expressible gene encodes GCaMP6, with two aspartic acid (D) -> cysteine (C) point mutations (dCys-GCaMP6; SEQ ID NO: 1).

[039] In a fifty-fourth embodiment, the method of detecting calcium levels in mammalian cells, uses a genetically encoded calcium sensor (GECS) based GCaMP-protein, encoded by an expressible gene in a recombinant lentivirus and stably expressed in cells. In a fifty-fifth embodiment, the expressible gene can be one of the GCaMP6 calcium sensors with two aspartic acid (D) -> cysteine (C) point mutations (dCys-GCaMP6; SEQ ID NO: 1), which showed increased signal and sensitivity to calcium in biochemical assays and in neuronal imaging, by decreasing the background and unspecific signal due to dead cells and exposure to extracellular calcium. We have used the GCaMP6-lentivirus successfully in human iPSCs using calcium imaging by microscopy as readout.

[040] Stable cell lines expressing GCaMPs for detection of calcium signals in cells have not been widely used in the neurobiological art, due to the reported toxicity of the GCaMP proteins of the first generation; transient transfections were used instead. GCaMP6s have not generally been used to generate a plate-based readout of calcium

levels in cells. GCaMP6s with the aspartic acid (D) -> cysteine (C) point mutations (dCys-GCaMP6; SEQ ID NO: 1) have also not generally been used. In a fifty-sixth embodiment, the calcium level detection method of the invention combines all three elements (stable expression allowing for clone selection, use of the improved GCaMP6s and introduction of GCaMP mutations). The calcium level detection method of the invention provides for the detection of calcium levels in cells without the need to use a calcium sensitive dye, thus providing cost savings and time savings and without the need to incubate the cells with the toxic dye anymore which might lead to false results due to cell stress, and making available a high throughput screen. The calcium level detection method of the invention can be used for cell imaging or in many assay formats, including the NMDAR assays of the invention.

[041] The invention provides assay and methods useful in screening for modulators of NMDARs, such as positive allosteric modulators ("PAMs") of NMDARs for use in treating or palliating schizophrenia or other NMDAR-based indications. This invention provides an assay to identify low molecular weight, brain-penetrant, NR2A-selective, positive allosteric modulators (PAMs) of the NMDAR. An advantage of PAMs is that they would enhance NMDAR activity exclusively when the receptor is activated by endogenously-released co-agonists (glutamate and D-serine or glycine), in response to physiological synaptic activity. An NR2A-specific PAM would allow engagement of a subpopulation of NMDARs thereby increasing the safety margin. Normalization of NMDAR activity in the brain of patients suffering from schizophrenia is predicted to mitigate positive, negative and cognitive symptoms thereby addressing unmet medical needs in schizophrenia. In a fifty-seventh embodiment, the method includes the steps of (a) transiently expressing NMDAR proteins NR1 and NR2, or the subunits, on the surface of a mammalian cell; (b) culturing the mammalian cell in a culture medium that contains an NMDAR ligand binding antagonist; and does not contain an NMDAR channel blocker; (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell; (d) measuring the calcium response by the mammalian cell; (f) contacting the mammalian cell with a test agent; (g) measuring the calcium response by the mammalian cell to the test agent; (i) contacting the mammalian cell with an NMDAR ligand site binder; and (j) again measuring the calcium response by the mammalian cell. An increase in calcium response by the mammalian cell identifies the test agent as a positive allosteric modulator of NMDARs for use in treating schizophrenia or other NMDAR-related indications.

[042] In a fifty-eighth embodiment, the test agent to palliate schizophrenia is further assayed by (b) measuring tyrosine phosphorylation of the NR2 subunit of the NMDAR of the cell in the presence and absence of the test agent, where an increase in tyrosine

phosphorylation of the NMDAR NR2 subunit in the presence of the test agent identifies an agent as a candidate compound to palliate schizophrenia. See, WO 2006/017748 (Decode Genetics EHF). In a fifty-ninth embodiment, assaying a test agent for electrophysiological effect upon interaction with a human CNS receptor can be done by
5 determining ligand-induced electrical current across said cell or membrane. See, U.S. Pat. No. 6,500,634 (Foldes *et al.*).

[043] In a sixtieth embodiment, transiently expressing NMDAR protein or protein subunit on the surface of the mammalian cell is accomplished by a transient transfection of the mammalian cell with a vector and expression of an NMDAR gene on the vector. In
10 a sixty-first embodiment, the vector can be a baculovirus vector containing an NMDAR gene, such as a BacMam vector containing an NMDAR gene.

[044] One advantage of the assay and methods of the invention is in discontinuing the use of ketamine in *in vitro* assays, which is an art-recognized problem to be solved. Cik *et al.*, *Biochem. J.* 296:877-83 (1993). To prevent NMDAR-mediated cytotoxicity,
15 previous studies have inhibited NMDAR activity by culturing cells in culture media containing ketamine or similar NMDAR channel blockers. These blockers are difficult to remove from the cells, even by extensive washing before the assay. Moreover, ketamine is a restricted substance, due to its abuse as a recreational drug.

[045] Another advantage of the assay and methods of the invention is speed. The
20 assay of the invention is efficient and can be used to measure NMDAR activity in a glycine-sensitive and glutamate-sensitive manner in a plate-based fluorescent assay in less than 24 hours. The method of the invention requires only a short incubation time, a total of about 16 hours in sixty-second embodiment. The setup of the assay of the invention does not require a clone selection step.

[046] Another advantage of the assay and methods of the invention is that the
25 expression and stoichiometry of NMDAR subunits is adjustable and titratable.

[047] Another advantage of the assay and methods of the invention is sensitivity to both glycine and L-glutamate. The assay and methods of the invention make available to neuroscientists and biochemists the rapid and flexible assessment of both glycine and
30 glutamate sensitivity of NMDAR in the same cells. Previous cell models have been sensitive to glutamate but not to glycine. Glycine is present at saturating amounts in culture media, and is produced and secreted by the mammalian cells themselves. In a sixty-third embodiment, the method of the invention includes a step of culturing cells in the presence of a weak glycine binding-site antagonist (MDL105,519), which can be
35 easily washed off the cells in a later step of the method. This results in a significant sensitivity to glycine ($EC_{50}=10\text{ }\mu\text{M}$) of our cells. In a sixty-fourth embodiment, the system

of the invention shows endogenously high levels of glutamate. L-glutamate sensitivity can be assayed by the addition of a weak glutamate binding site antagonist.

[048] Another advantage is the increased sensitivity of the assay due to a lowering of the background fluorescence caused by the calcium 6 dyes.

5 **[049]** Another advantage is the removal of the dye loading step, which is toxic for some cell types and further increases the background signal and hence decreases sensitivity. Another advantage is that there is no need to add probenecid, which can also interfere with assay of the invention or similar assays.

[050] Another advantage of the assay and methods of the invention is that it makes
10 available to neuroscientists and biochemists the measurement of D-serine effect on NMDARs. D-serine -binds to the same NMDAR binding site as glycine. L-serine is much more abundant than D-serine, and the high L-serine levels have previously made it difficult to measure D-serine levels with classical biochemical methods. The system of the invention now makes available to neuroscientists and biochemists readout for D-
15 serine levels generated in biochemical reactions or cell supernatants, even in the presence of L-serine. NMDARs are ~200-fold less sensitive to L-serine than D-serine , which now makes available the analysis of D-serine levels in the presence of L-serine.

[051] An unexpected result of the assay and methods of the invention is that it is necessary to block ligand binding of native NMDAR during the culturing step. In one
20 aspect, data generated in the practice of the invention show that it is not sufficient to only block NMDAR-mediated toxicity in the cells (e.g., with ketamine, as in previous assays). It is also necessary to block ligand binding to the native receptors during the mammalian cell culture. The practice of the invention does not result in a complete rescue of cell viability, but does result in a much better signal in the activity assay.

25 **[052]** In another aspect, the assay of the invention can be used to identify inhibitors of NMDAR (competitive, noncompetitive, and allosteric).

[053] In another aspect, the method can be used to assess the effect of the test agent at different activation levels of NMDAR (positive allosteric modulator assays are run at sub-maximal ligand concentrations, whereas inhibitor assays are run at EC80-100 ligand
30 concentration).

[054] In another aspect, the assay can be used to identify compounds that show ligand-specificity, i.e. they only work in the presence of on ligand but not the other.

DESCRIPTION OF THE DRAWINGS

35 **[055]** FIG. 1 is an immunoblot showing the expression of NMDAR in mammalian cells using baculovirus. HEK293 cells in the presence of 1 mM ketamine were transduced with different amounts of baculovirus vector encoding human NR1 and NR2A. The cells were

harvested 16 hours after transduction. NR1 and NR2A protein levels were analyzed by immunoblotting. Thus, FIG. 1 shows NR1 and NR2A protein levels in HEK293 cells transduced with baculovirus. Representative images are shown. This figure supports the finding that the method of the invention is titratable.

5 **[056]** FIG. 2 is a model showing the prior art lack of NMDAR activity in functional assay after protection with MK801 or ketamine. NMDAR-ligands contained or secreted by the cells into the media activate NMDAR and lead to excitotoxicity. Protection with MK801 or ketamine prevent excitotoxicity, but are difficult to wash out and do not prevent occupation of the ligand binding sites with endogenous ligand, both of which prevent
10 NMDAR activity in functional assays.

[057] FIG. 3 is a model of the protection facilitated sensitivity of NMDAR activity to glycine ("Protection with glycine binding antagonist MDL105,519 (Glycine-sensitive mode)") and glutamate ("Protection with glutamate binding antagonist CGP070667 (Glutamate-sensitive mode)"). The addition of ligand binding site antagonists protects
15 NMDAR by occupying the ligand binding site and preventing the binding of endogenous ligands. After washout of the ligand binding site antagonist, the ligand binding sites become accessible for exogenously added ligand, thus facilitating ligand-sensitivity.

[058] FIG. 4 is a plasmid map of the Gateway compatible BacMam vector prior to recombination to produce final expression vectors.

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

[059] "7-CKA", "7-CTKA", or "7-chlorokynurenic acid" is 7-chloro-4-oxo-1H-quinoline-2-carboxylic acid, is a weak competitive NMDAR glycine binding site antagonist, CAS
25 Number 18000-24-3. 7-CKA is commercially available from Sigma-Aldrich, St. Louis MO USA, and from Tocris Bioscience, Minneapolis MN USA.

[060] "AAM077" is [[[1S)-1-(4-Bromophenyl)ethyl]amino](1,2,3,4-tetrahydro-2,3-dioxo-5-quinoxaliny)methyl]phosphonic acid tetrasodium hydrate, CAS Registry Number 459836-30-7, a NR2A-selective NMDAR antagonist. See, Frizelle P *et al.*, *Mol.*
30 *Pharmacol.* 70: 1022 (2006). AAM077 is commercially available from Calbiochem, Billerica MA USA.

[061] "AP5" (2-amino-5-phosphonopentanoic acid, APV) is a competitive glutamate binding site antagonist. The CAS Registry Number is 76326-31-3 AP5 is commercially available from Sigma-Aldrich, St. Louis MO USA, and from Tocris Bioscience,
35 Minneapolis MN USA. In the assay of the invention (TABLE 4), AP5 was not sufficient as an NMDAR ligand binding site antagonists to protect the cells from excitotoxicity.

- [062] "BacMam", a Baculovirus gene transfer into Mammalian cells system, is a use of modified insect cell virus (baculovirus) as a vector to deliver and express genes in mammalian cells. See, U.S. Pat. No. 5,871,986 (Boyce), Hofmann & Strauss, "Baculovirus-mediated gene transfer in the presence of human serum or blood facilitated by inhibition of the complement system", *Gene Therapy* 5 (4): 531–536 (1998); Boyce & Bucher, "Baculovirus-mediated gene transfer into mammalian cells", *Proc. Natl. Acad. Sci., U.S.A.* 93(6): 2348–52 (1996); Ames *et al.*, "BacMam technology and its application to drug discovery". *Expert Opin. Drug Discov.* 2(12): 1669–1681 (2007). BacMam can be purchased from Thermo Fisher Scientific, Waltham MA USA.
- 10 [063] "A "bacmid" is a baculovirus shuttle vector that can be propagated in both *E. coli* and insect cells.
- [064] A "baculovirus vector" is a covalently closed circular double stranded polynucleotide, which can accommodate the large fragments of foreign DNA. Baculovirus vectors are based upon *Baculoviridae* viruses, which infect arthropods as their natural
- 15 hosts. The baculovirus vectors mainly used as expression vectors are based upon *Autographa californica* nucleopolyhedrovirus (NPV), which was isolated from alfalfa looper larva. Examples of commercially available baculovirus vectors include the BacMam system (see above), the Baculovirus Expression Vector System (from BD Biosciences, San Diego CA USA) and Baculovirus Expression System – BacPAK (from
- 20 Clontech Laboratories, Inc., Mountain View CA USA). A recombinant baculovirus vector can contain one or more nucleotide sequence of a human NMDAR NR1 subunit and/or NR2 subunit together with additional sequences capable of directing the synthesis of the human NMDAR subunit in cultures of transiently transfected mammalian cells. See, EP0672140B1 (Merck Sharp & Dohme, Ltd.).
- 25 [001] A "calcium sensor protein" is a protein that can be expressed inside living (viable) cells and responds in a measurable way to changes in calcium concentration. Examples of calcium sensor proteins include the GCaMPs.
- [002] "Calcium 6 dye" is a proprietary fluorophore that provides a high quantum yield compared to other calcium indicators, for use with a FLIPR® calcium assay kit. FLIPR®
- 30 Assay Kits are commercially available from Molecular Devices, LLC, Sunnyvale CA USA. Similar calcium dyes for use in FLIPR® Assay Kits from Molecular Devices include calcium 5 dye, calcium 4 dye and calcium 3 dye.
- [003] A "cell-permeable calcium indicator" is a chemical compound that can pass from culture media or solutions through the membranes of cells, where they respond in a
- 35 measurable way to changes in calcium concentrations in living or viable cells. Examples of cell-permeable calcium indicators are fura-2, Indo-1, calcium 3 dye, calcium 4 dye, calcium 5 dye and calcium 6 dye. Methods of measuring changes in calcium

concentration in the mammalian cell are known in the neurobiological arts. For example, primary cultures of rat cerebellar granule cells loaded with the fluorimetric indicator fura-2 were used to measure changes in $[Ca^{2+}]$ elicited by NMDA and its co-agonist glycine.

See, WO 95/21612 (NPS Pharma, Inc.).

- 5 **[004]** "CellTiter-Glo®", a Luminescent Cell Viability Assay, is a method of determining the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells. The CellTiter-Glo® Assay is available from Promega Corporation, Madison WI USA.

- [005]** "CGP039653" is an NMDAR ligand. See, Auberson YP *et al.*, *Bioorg. Med. Chem. Lett.* 12: 1099–1102 (2002).

[006] "CGP070667" is ((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)methyl)phosphonic acid. See, Auberson YP *et al.*, *Bioorg. Med. Chem. Lett.* 12: 1099–1102 (2002).

- [007]** "CIQ" is (3-chlorophenyl)(6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-3,4-dihydroisoquinolin-2(1H)-yl)methanone, CAS Registry Number 486427-17-2. See, Zhang X *et al.*, *British J. Pharmacol.* 171(16): 3938–3945 (August 2014). CIQ is commercially available from Tocris Bioscience, Minneapolis MN USA.

[008] "CPP" is 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid, a selective NMDAR antagonist. CPP can be purchased as a (±)-CPP solid from Sigma-Aldrich Corp., St. Louis MO USA.

- 20 **[009]** "CP101606", "CP-101,606" or "traxoprodil" is (1S, 2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, a noncompetitive antagonist of NMDARs containing the NR2B subunit. See, Menniti FS *et al.*, *Neuropharmacol.* 39(7):1147-55 (27 April 2000). CP-101,606 is produced by Pfizer (Groton CN USA).

- [010]** dCys-GCAMP6s (SEQ ID NO: 1) is a dye showing similar sensitivity as calcium6 dye. The mutations introduced into dCys-GCAMP6s increase sensitivity as compared to Calcium6 dye. The specific mutations render dCys-GCAMP6s insensitive to calcium under oxidative conditions (extracellular environment). This results in a reduction of unspecific signal due to loss of membrane integrity (*e.g.*, dead cells). Cells containing dCys-GCAMP6s show similar to higher sensitivity and higher signal range than cells loaded with Calcium6 dye. The advantages of using dCys-GCAMP6s include (a) cost; (b) time; (c) sensitivity; (d) flexibility; and (e) opportunities for multiplexing

- [011]** "Doxycycline" is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide, CAS Registry Number 564-25-0, an antibiotic that is used in the treatment of a number of types of infections caused by bacteria and protozoa, and for prophylaxis against malaria.

“FDSS” is a Functional Drug Screening System. FDSS/ μ CELL is a kinetic plate reader with an integrated dispensing head and imaging-based detector, allowing for simultaneous dispensing into 96/384-well plates and simultaneous detection of the kinetics of the fluorescence or luminescence intensity. FDSS/ μ CELL can be purchased from Hamamatsu Photonics K.K., Hamamatsu City, Japan. Hamamatsu also provides a 1536 well format. The system allows for online liquid addition and is compatible with classical fluorescent dyes for calcium imaging.

[012] “GCaMP” is a genetically encoded calcium indicator (GECI), created from a fusion of green fluorescent protein (GFP), calmodulin, and M13, a peptide sequence from myosin light chain kinase. Nakai *et al.*, *Nature Biotechnol.* 19:137-141 (2001). GCaMPs have been modified to improve the range of the fluorescence signal, resulting in GCaMP3 (Tian *et al.*, *Nat. Methods* 6:875-881 (2009)) through GCaMP8 (Ohkura *et al.*, *PLoS ONE* 7(12): 051286 (2012)). Red fluorescence GECIs have also been developed. Zhao *et al.*, *Science* 333:1888-1891 (2011).

[013] “GCaMP6” is a calcium sensor (GECs) based GCaMP protein. See, Chen *et al.*, *Nature* 499(7458): 295-300 (18 July 2013). GCaMP6 and vectors containing the GCaMP6 gene are commercially available from Addgene (Cambridge MA USA). In the method of the invention, dCys-GCaMP6s shows similar sensitivity as calcium 6 dye and higher signal range. The mutations increase sensitivity to calcium by rendering the protein probe insensitive to calcium under oxidative conditions (such as extracellular environment), which leads to reduction of unspecific signal due to loss of membrane integrity (as in dead cells).

[014] “Glutamate”, “L-glutamate” or “Glu” is a naturally-occurring amino acid. NMDARs have a glutamate binding site.

[015] “Glycine” or “Gly” is a naturally-occurring amino acid. NMDARs have a glycine binding site.

[016] “GNE-6901” is 7-(4-fluorophenoxymethyl-3-[trans-2-hydroxymethyl)cyclopropyl]-2-methyl-5H-[1,3]thiazolo[3,2- α]pyrimidin-5-one (enantiomer 2), as described in WO2015/052226 (Genentech, Inc.).

[017] “HBSS” is Hank's Balanced Salt Solution, a balanced salt solution rich in bicarbonate ions, made to a physiological pH (roughly 7.0-7.4) and salt concentration, maintain mammalian cells' pH and osmotic balance. Originally prepared by Hanks & Wallace, *Proc. Soc. Exp. Biol. Med.* 71:196-200 (1949), HBSS is now available in several formulations and formats. HBSS is often used for washing cells, as a diluent, or as an inorganic base for standard media preparations. HBSS can be purchased from commercial sources, including from Thermo Fisher Scientific, Waltham MA USA.

- [018] "HEK cells", "HEK293", "HEK-293" or "293 cells" are Human Embryonic Kidney 293 cells, which are derived from cells originally generated in 1973 by transformation of cultures of normal human embryonic kidney cells with sheared adenovirus 5 DNA. Graham *et al.*, *J. Gen. Virol.* 36(1): 59–74 (1977). HEK cells are available from commercial sources, including from the American Type Culture Collection (ATCC), Manassas VA USA.
- [019] A "high-throughput screen" ("HTS") is a drug-discovery process widely used in the pharmaceutical industry that uses automation, robotics, data processing and control software, liquid handling devices, and sensitive detectors to quickly assay the biological or biochemical activity of a large number of drug-like compounds. See, "High-Throughput Screening Challenges". *Genetic Engineering & Biotechnology News. Drug Discovery Roundtable Discussion* 28(14): 26–27. (Mary Ann Liebert, 01 August, 2008).
- [020] "Ifenprodil" is 4-[2-(4-benzylpiperidin-1-yl)-1-hydroxypropyl]phenol, a selective inhibitor of NMDARs composed of the NR1/2B subunits. See, Reynolds & Miller, *Mol. Pharmacol.* 36(5): 758–65 (1989).
- [021] "Ketamine" is the NMDAR channel blocker/non-competitive inhibitor (RS)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone. Ketamine is a dissociative psychedelic with antidepressant properties used as an anesthesia in humans and animals, and is also used recreationally for its effects on the central nervous system, and thus regulated as a Controlled Substance in many countries. Ketamine's use as a recreational drug has been implicated in several deaths worldwide.
- [022] "L701,324" is 7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1H)-quinolinone, a selective NMDAR glycine site antagonist. Obrenovitch & Zilkha, *British J. Pharmacol.* 117: 931-937 (1996).
- [023] "Lentivirus vectors" or "lentiviral vectors" are a type of retrovirus that are adapted as gene delivery vehicles (vectors) in the biochemical arts and that can infect both dividing and non-dividing cells. For methods of using lentivirus vectors, see Green & Sambrook, *Molecular Cloning: A Laboratory Manual, Fourth Edition* (Cold Spring Harbor Press, Plainview, NY, 2012).
- [024] "MDL105,519" (CAS Registry Number 161230-88-2) is a competitive NMDAR glycine binding site antagonist and an inhibitor of NMDAR-mediated responses *in vitro* and *in vivo*. MDL105,519 is commercially available from PerkinElmer, Waltham MA USA.
- [025] "Memantine" is the NMDAR channel blocker 3,5-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-amine (CAS Registry Number 19982-08-2). Memantine is commercially available from several pharmaceutical companies, including Forest Laboratories, New York NY USA, as Namenda®.

- [026] "MK801" or "dizocilpine" is the NMDAR channel blocker, [5R,10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, CAS Registry Number 77086-21-6. See, U.S. Pat. Nos. 4,399,141 (Anderson *et al.*) and 4,477,668 (Bender *et al.*). MK801 has been used as a recreational drug. MK801 is available from commercial sources, including from Calbiochem, Billerica MA USA.
- [027] A "modulator of an N-methyl-D-aspartate receptor (NMDAR) function" is a compound that modulates the activity of an NMDAR protein or NMDAR subunit. A modulator of NMDAR function can be an uncompetitive channel blocker; a non-competitive antagonist, partial agonist or agonist; or a competitive antagonist, partial agonist or agonist. See, examples in Van Dongen AM, editor. *Biology of the NMDA Receptor*. (CRC Press/Taylor & Francis, Boca Raton FL USA, 2009). A modulator of NMDAR function can be determined by the method or assay of the invention.
- [028] "N-methyl-D-aspartate receptor" (also known as "NMDA receptor", "NMDAR" or "NMDAR protein") is a glutamate receptor and ion channel protein found, *e.g.*, in nerve cells. NMDAR includes seven different receptor subunits. Paoletti *et al.*, *Nature Rev. Neurosci.* 1: 383–400 (2013). NMDAR protein comprises the GRIN1 subunit, which can combine with GRIN2 subunits (GRIN2A, GRIN2B, GRIN2C, and GRIN2D) and/or GRIN3 subunits (GRIN3A and GRIN3B). Combinations of these subunits can form either di-heteromeric (*e.g.*, 2 units of GRIN1 and 2 GRIN2A subunits) or more complex tri-heteromeric (*e.g.*, 2 units of GRIN1 and 1 GRIN2A, 1 GRIN2B) receptor complexes. NMDARs mediate the transfer of electrical signals between neurons in the brain and in the spinal column. For electrical signals to pass, the NMDAR must be open. To remain open, glutamate and glycine must bind to the NMDA receptor. An NMDAR that has glycine and glutamate bound to it and has an open ion channel is "activated."
- [029] "NMDAR antagonists" are a class of anesthetics that work to antagonize, deactivate or inhibit the action of NMDAR. Chemicals that block the function of the NMDAR are called antagonists. NMDAR antagonists fall into four categories: competitive antagonists (which bind to and block the binding site of the neurotransmitter glutamate); glycine antagonists (which bind to and block the glycine site); noncompetitive antagonists (which inhibit NMDARs by binding to allosteric sites); and uncompetitive antagonists (which block the ion channel by binding to a site within it). See, Kim AH *et al.*, "Blocking Excitotoxicity". In Marcoux FW & Choi DW. *CNS Neuroprotection*, pp. 3–36 (New York: Springer, 2002).
- [030] "NMDAR channel blockers" are uncompetitive or non-competitive NMDAR antagonists that block the open ion channel, particularly after activation by agonists. Examples of NMDAR channel blockers include ketamine and dizocilpine (MK801). To prevent cell death induced by the expression of the recombinant human NMDA

receptors, some have found it advisable to incorporate ketamine at an appropriate concentration into the culture medium to prevent cell death induced by the expression of the recombinant NMDARs. See, British patent application GB 2291647A (Merck Sharp & Dohme, Ltd.) and WO 2006/017748 (Decode Genetics EHF). Some NMDAR channel
5 blockers, such as ketamine, are recreational drugs used for their dissociative, hallucinogenic, and euphoriant properties.

[031] "NMDAR glutamate binding site antagonists" are compounds that bind to glutamate binding site on NMDARs. Glutamate ligand binding site antagonists include the protective compound CGP070667.

10 **[032]** "NMDAR glycine binding site antagonists" are compounds that bind to glycine binding site on NMDARs. Glycine binding site antagonists include the protective compound MDL105,519.

[033] "NMDAR ligand binding site antagonist" is a compound that binds to the glutamate binding site or glycine/D-serine binding site of NMDAR to antagonize NMDAR
15 activity. The NMDAR ligand binding site antagonist can be MDL105,519 or CGP070667.

[034] "NMDAR ligand site binder" is a compound that binds to the glutamate or glycine/D-serine binding site of NMDAR to affect NMDAR, particularly in the later stages of the method of the invention. NMDAR ligand site binder can bind to NMDAR glutamate sites or NMDAR glycine sites, and can be positive or negative effectors of NMDAR
20 activity. The NMDAR ligand site binder can be CGP070667, AAM077, 7-CTKA (7-CKA), MDL105,519, L701,324, CGP039653 or CPP.

[035] "NR1/2A", "NR1/NR2A" or "GNIR1/NR2A" is an NMDAR comprising only subunits NR1 (glycine binding NMDA receptor subunit 1; GluN1) and NR2A (glutamate binding NMDA receptor subunit 2; GluN2A) subunits.

25 **[036]** "NR1/2B", "NR1/NR2B" or "GNIR1/NR2B" is an NMDAR comprising only subunits NR1 (glycine binding NMDA receptor subunit 1; GluN1) and NR2B (glutamate binding NMDA receptor subunit 2; GluN2B) subunits.

[037] "NR1/2B", "NR1/NR2D" or "GNIR1/NR2D" is an NMDAR comprising only subunits NR1 (glycine binding NMDA receptor subunit 1; GluN1) and NR2D (glutamate binding
30 NMDA receptor subunit 2; GluN2D) subunits.

[038] "Plasmids" are small DNA molecules within a cell that is physically separated from a chromosomal DNA and can replicate independently. Plasmids are most commonly found in bacteria as small, circular, double-stranded DNA molecules; however, plasmids are sometimes present in eukaryotes. In U.S. Pat No. 6,500,634
35 (Foldes *et al.*), EXAMPLE 3, for the transient expression of NMDAR in HEK293 cells, approximately 2 µg DNA (as plasmids pcDNA 1-Amp/hNR2A or pcDNA 1-Amp/hNR 1-3C) was transfected per 10⁵ HEK293 cells, by lipofectin-mediated DNA transfection

- according to the manufacturer's (Life Technologies Inc., Gaithersburg MD USA) specifications. In co-expression experiments, *i.e.* for heteromeric expression of NR2A-1 and NMDAR1-3C, the HEK293 cells were similarly transfected with 3 µg of a DNA mixture containing pcDNA1-Amp/hNR2A and pcDNA1-Amp/hNR1-3C. Briefly, HEK293
- 5 cells were plated at a density of 10⁵ cells/dish and then grown for 24 hours in 10% FBS-supplemented MEM medium (Life Technologies Inc., Gaithersburg MD USA). The medium was then removed and cells were washed in OPTI-MEM I medium (Life Technologies Inc., Gaithersburg MD USA) lacking FBS, prior to transfection. A transfection solution (100 µl) containing 5-7.5 µl of lipofectin and DNA was then applied
- 10 to the cells. After incubation for six hours at 37°C, cells were washed and then allowed to grow for 36-48 hours in 10% FBS-supplemented MEM medium containing 50 µM glutamate binding site antagonist AP5 prior to electrophysiological recording. Other slightly different protocols, using different reagents like FuGene6, are known to those of skill in the art.
- 15 **[039]** "Probenecid" is 4-(dipropylsulfamoyl)benzoic acid, CAS Registry Number 57-66-9, sold under the brand name Probalan.
- [040]** "QNZ46" is 4-[6-methoxy-2-[(1E)-2-(3-nitrophenyl)ethenyl]-4-oxo-3(4H)quinazolinyl]benzoic acid. See, Mosley *et al.*, *J. Med. Chem.* 53 5476 (2010); Hansen & Traynelis, *J. Neurosci.* 31: 3650 (2011). QNZ46 is commercially available from
- 20 Tocris Bioscience, Minneapolis MN USA.
- [041]** "RO25-6981" is (αR,βS)-α-(4-Hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol, a potent and selective activity-dependent blocker of NMDA receptors containing the NR2B subunit. See, Fischer *et al.*, *J. Pharmacol. Exp. Ther.* 283: 1285 (1997). RO25-6981 is commercially available from Tocris Bioscience, Minneapolis MN
- 25 USA.
- [042]** "TCN201" is 3-chloro-4-fluoro-N-[4-[[2-(phenylcarbonyl)hydrazino]carbonyl]benzyl]benzenesulfonamide, an NMDAR antagonist selective for NR1/2A over NR1/2B-containing receptors. TCN201 is an allosteric negative modulator of NMDARs. TCN201 is commercially available from Tocris Bioscience,
- 30 Minneapolis MN USA. TCN201 selectively blocks N2A-containing NMDARs in a N1 co-agonist dependent, but non-competitive manner. Edman S *et al.*, *Neuropharmacology* (2012). In the assay of the invention (TABLE 4), TCN201 was not found to be sufficient as an NMDAR ligand binding site antagonist to protect the cells from excitotoxicity.
- [043]** A "test agent" is an agent that is suspected of being a modulator of NMDAR
- 35 function and thus a candidate for testing by the method of the invention. A test agent can be suspected of being a modulator of NMDAR function based upon its activity in a

different assay than the assay of the invention, based upon its chemical structure, or for any other reason.

- [044] A “viable” cell is a cell, whether *in vivo* or *in vivo*, that is alive. Viability of a cell can be determined by any means known to those in the neurobiological or biochemical arts, such as by quantitation of the ATP present in cells, which is an indicator of metabolically active cells. One assay for quantitation of the ATP present in cells is a CellTiter-Glo® assay. See, TABLE 2.

Utility

- [045] NMDARs have been studied by neuroscientists and biochemists because of their involvement in synaptic plasticity and cognition. Genetics and functional studies have implicated glutamate and NMDARs in schizophrenia. Gordon JA, “Testing the glutamate hypothesis of schizophrenia”, *Nat. Neurosci.* 13, 2-4 (2010). NMDARs have been implicated in other nervous system disorders such as epilepsy, stroke, pain, addiction, depression and Alzheimer’s disease. NMDARs may also be targets for diseases in other organ systems such as diabetes (Marquard *et al.*, *Nature Medicine*, 21(4): 363 (2015)), in regulation immune function (Simma *et al.*, *Cell Commun. Signal.*, 12: 75 (2014); Farooq *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 307(7): G732-40 (2014)), tendinopathy, IBS (Camilleri *et al.*, *J. Pediatr. Gastroenterol. Nutr.*, 54(4):446-53 (2012)), and glaucoma (Nowak *et al.*, *Pol. J. Pathol.*, 65(4), 313-21 (2014)). The ability to study the different receptor subunits, in a high-throughput fashion is of interest to neuroscientists, biochemists and other biologists.

- [046] Ectopic expression of functional NMDARs in non-neuronal cells is known to cause excitotoxicity and cell death. The toxic effect of NMDAR correlates with expression level. The toxic effect had previously been overcome by the treatment with NMDAR channel blockers, which interfere with NMDAR activity in functional downstream assays.

- [047] Many of the pharmacological and receptor characterization studies to date have been performed in low or medium throughput electrophysiological assays such as manual patch clamp, port-a-patch, Qpatch, Ionworks Barracuda or Ionworks Quattro. See other examples in Cao *et al.*, *Assay and Drug Development Technologies* 8:766-80 (2010). By contrast, our assay recapitulates these findings via a calcium readout enabling the study of thousands to millions of compounds. The development of such an assay is timely given the advances in receptor pharmacology, the elucidation of its structure, and the genetic link of different subunits and its co-agonists to disease.

- [048] Cell-based electrophysiological assays can be useful in conjunction with ion channel patch clamp technology. See, Wickenden, Priest, Erdemli (2012) “Ion Channel Drug Discovery: Challenges and Future Directions”, *Future Medicinal Chemistry* 4:661-

79, which discloses that due to limitations in high throughput assay technologies that support screening and lead optimization, ion channels remain significantly under-exploited as therapeutic targets. See also, Cao *et al*, *Assay and Drug Development Technologies* 8:766-80 (2010), which discloses the visualization of recording quality and pharmacological characterization. In one aspect of the invention, an R-script for quantitative analysis of the data has been developed.

[049] Historically, targeting NMDARs have been challenging. Many antagonists failed clinical trials due to dose-limiting side effects and, the identification of agonists posed significant risk as over-stimulation of this receptor is implicated in several pathologies. However, in the past few years there has been a lot of progress in understanding the receptor and its complex biology. The fact that the receptor complex can be composed of different subunits with different properties led to the discovery of small molecules that are selective for some subunits over others. Ogden & Traynelis, *Trends Pharmacol. Sci.* 32(12): 726-33 (December 2011). Targeting specific subunits can de-risk mechanism based toxicities associated with hitting all receptor types.

[050] The elucidation of the structure of the NMDAR complex has helped understand the different mechanism of these molecules as well as to generate hypothesis on how to target and modulate different parts of the complex. Lee C-H *et al.*, *Nature* 511: 191–197 (10 July 2014); Karakas & Furukawa, *Science* 344(6187): 992-997 (30 May 2014). For example, two sites for allosteric modulation have been proposed, either the ligand-binding domain or the N-terminus domain. Moreover, molecules can bind to the interface between different subunits, domains, or linker regions opening the opportunity to find diverse mechanisms to modulate these receptors. Ogden & Traynelis, *Trends Pharmacol. Sci.* 32(12): 726-33 (December 2011); Santangelo RM *et al.*, *Expert Opin. Ther. Pat.* 22(11):1337-52 (November 2012).

[051] The inventors wanted to identify and develop NMDAR modulators, to treat or palliate schizophrenia and other NMDAR-related diseases. We recognized the challenge developing such NMDAR modulators, given the experience of others in the neurobiological art. Thus, our first efforts were to develop appropriate cellular model systems for studying NMDAR activity that would overcome the limitations of previously used models. We intended that the cellular model systems would be robust high-throughput screening (HTS) assays; could be used for in-depth studies of the mechanisms of action of the test compounds; and could be used to identify compounds with differential activity profiles, for subsequent *in vivo* studies to identify optimal compounds.

[052] A consistent finding from post-mortem schizophrenia brain analyses are correlates that point to dysfunction of the parvalbumin (PV)-positive class of inhibitory

interneurons (INs). PV-INs regulate excitatory circuit activity and play a critical role in establishing the brain's excitatory/inhibitory balance. PV-INs express higher levels of NR2A-containing NMDARs than any other sub-type and are particularly vulnerable to deficits in NR2A activity. Post-mortem schizophrenia brain analyses also show a
5 decrease in dendritic spine density in glutamatergic neurons, enhanced neuregulin-erb4 signaling (known to suppress NMDAR function), reduced levels of NR2A-containing NMDARs and a decreased density of NR2A-positive GABAergic interneurons. These studies highlight a critical role for glutamate signaling, likely via NR2A-containing NMDARs in SZ pathophysiology. Enhancement of NMDAR activity with agents that
10 target the glycine modulatory site directly (e.g. glycine, D-serine, alanine) or indirectly (sarcosine) in chronic schizophrenia patients who were also receiving antipsychotics yielded promising results. The improvement of negative symptoms is substantial and the effects on positive symptoms and cognition, while modest, are significant. These studies provide proof of principle that enhancing NMDAR function can reduce symptoms in
15 patients suffering from schizophrenia. The restoration of NMDAR function in parvalbumin positive interneurons would be expected to rebalance cortico-striatal circuitry.

[053] The invention provides a different approach to studying the pharmacology of NMDARs. The unbiased identification of weak glycine and glutamate site antagonists that protect cells from NMADR toxicity and the ability to readily remove these compounds
20 were important in the development of the assay of the invention. These compounds compete out the exogenous and saturating levels of ligand (L-glutamate or glycine) that maintain the receptor in an activated, presumably desensitized state. Once these compounds are removed, the glycine/D-serine site is no longer saturated, enabling the study of different co-agonist concentrations on calcium signaling with much less
25 background interference.

[054] The assay of the invention is biologically relevant. We were able to recapitulate the EC_{50} s for the agonist glutamate and the co-agonists glycine or D-serine . We were able to faithfully recapitulate the effects of several known NMDARs inhibitors and allosteric modulators in our assay. See TABLE 17.

30 **[055]** The assay of the invention also shows the expected sensitivity to magnesium. With depolarized membrane potentials, the assay of the invention becomes less sensitive to magnesium. See, TABLE 11.

[056] Such an opportunity further highlights the need of a high-throughput approach to identify small molecules with broad and distinct modes of action. Herein, we
35 demonstrated how our assay could be used to study inhibitors as well as allosteric modulators with distinct modes of action.

- [057]** Having the ability to study different NMDA receptor complexes, in particular with respect to its co-agonist, is relevant for many different neurological diseases. In addition to subunit selectivity, one way to overcome potential issues with targeting the NMDAR receptor directly, has been to modulate levels of its co-agonists glycine and D-serine in particular in disorders like schizophrenia where glutamate hypofunction is thought to be central to the disorder. Balu DT & Coyle JT, *Curr. Opin. Pharmacol.* 20:109-15 (February 2015); Levin R *et al.*, *J. Psychiatr. Res.* 61: 188-95 (February 2015). The rationale is that subtler activation of the receptor can be achieved by modulation of glycine and D-serine as opposed to targeting glutamate itself. Moghaddam B & Javitt D, *Neuropsychopharmacol.* 37(1):4-15 (January 2014); Hashimoto K *et al.*, *Eur. Arch. Psychiatry Clin. Neurosci.* 263(5):367-77 (August 2013). Supporting this evidence, low levels of D-serine have been associated with schizophrenia and several genetic studies support this hypothesis. For example, serine racemase and D-amino acid oxidase (DAAO), both regulators of D-serine production, have been associated with schizophrenia and polymorphism in GRIN2A strongly correlated with levels of D-serine. Balu DT & Coyle JT, *Curr. Opin. Pharmacol.* 20:109-15 (February 2015). D-serine levels have also been implicated in pain, Alzheimer's disease, and depression. Lefèvre Y *et al.*, *Neurosci. Lett.* 603: 42-7 (31 August 2015); Wu S *et al.*, *Curr. Alzheimer Res.* 4(3):243-51 (July 2007); Madeira C *et al.*, *Transl. Psychiatry* e561 (05 May 2015).
- [058]** The method of the invention uses BacMam to introduce different receptor subunits. The use of a BacMam vector, in combination with the use of weak glycine and glutamate site antagonists, allows neuroscientists and biochemists to study distinct pharmacological activities of small molecules such as the identification of co-agonist glycine or D-serine molecules vs. glutamate as well as to test the effect of small molecules in different receptor subunits (di-heteromeric or tri-heteromeric).
- [059]** The use of recombinant baculovirus vectors in which a mammalian-active promoter is present enables transient transfection of mammalian cells. See, Ames *et al.*, *Expert Opin. Drug Discov.* 2: 1669-81 (2007). Baculovirus vectors provide a gentle, titratable way of transfecting mammalian cell lines and primary cells. For assays in which expression from multiple different subunits are compared, expression from individual baculovirus vectors offers the advantage of individually titratable expression and the ease of switching protein subunits using individually encoding viruses. See, FIG. 1.
- [060]** Alternatives to the BacMam system include the traditional lipofectamine or Fugene-based transient transfection systems. Expression of the NMDAR may also be accomplished by a variety of different promoter-expression systems in a variety of different host cells. See, e.g., EP 0 672 140 B1 (Merck Sharp and Dohme, Ltd.), which discloses transiently transfected cells containing NMDAR. The eukaryotic host cells

suitably include yeast, insect and mammalian cells. Suitable mammalian host cells include rodent fibroblast lines, e.g., mouse Ltk⁺, Chinese hamster ovary (CHO) and baby hamster kidney (BHK); HeLa; and HEK293 cells.

[061] The assay and methods of the invention provide for rapid comparisons of
5 different NMDAR subunits. Thus, the assay and methods of the invention now make available to neuroscientists and biochemists a way to measure the effects of agonists for both the glycine binding site (e.g., glycine or D-serine) and the glutamate binding site (e.g., glutamate or NMDA) in different combinations. The assay and methods of the invention do not use channel blockers such as ketamine or MK-801 to mitigate against
10 cellular toxicity and maintaining the ligand binding site in a ligand-free state. Instead, the assay and methods of the invention leverage the use of weak NMDAR glycine binding site antagonists or weak NMDAR glutamate binding antagonists, which now can be identified in an unbiased screen.

[062] These weak NMDAR glycine binding site antagonists or weak NMDAR glutamate
15 binding antagonists are readily washed off, resulting in the ability to measure reliable calcium traces following activation with different concentrations of ligand and/or ligand combinations. The use of weak antagonists now makes available to neuroscientists and biochemists a way to not only maintain cells alive during the assay, but also to study the activation of NMDARs by different ligands, with sensitivity to both D-serine /glycine and
20 glutamate/NMDA. The assay and methods of the invention provide neuroscientists and biochemists with the ability to investigate the effects of small molecules on the activation of NMDARs using different ligands and ligand binding sites.

[063] The assay system of the invention replicates the pharmacology of NMDAR in response to known antagonists, allosteric modulators, as well as sensitivity to
25 magnesium.

[064] Previous cell based approaches to study NMDARs are often confounded and hindered by the high background levels of endogenous glutamate and glycine. The assay of the invention removes this burden, allowing neuroscientists and biochemists to more faithfully observe the effects of small molecules on the agonist, co-agonist, or both
30 sites hence enabling the identification of molecules that may work via different mechanisms. The ability to rapidly and in large scale study the biology of NMDARs through the method of the invention now makes available to neuroscientists and biochemists the identification of novel therapeutics, whose discovery would have otherwise have been confounded by the limitations of previous cell-based approaches.

EXAMPLES

EXAMPLE IPreliminary efforts to express NMDAR in mammalian cells

5 [065] Because NMDAR expression in cells *in vitro* is cytotoxic, we the inventors began efforts to develop an NMDAR assay that does not have the limitations of currently available models, which have (a) high expression levels of NMDAR NR2 subunit; (b) a random ratio of NMDAR subunits; (c) a need to use ketamine or similar strong NMDAR channel blockers for protection excitotoxicity, and (d) a lack of sensitivity to glycine/D-serine (and a limited sensitivity to glutamate) in a plate based assay, due to the high
10 levels of glycine in cell media, including glycine and other amino acids secreted by dying cells in culture.

[066] One set of attempts used the Neuro2A mouse cell line, which has a membrane potential of ~ -70 mV. Attempts to accomplish the transient transfection or constitutive
15 expression were unsuccessful. An attempt at the positive selection of stable clones was also unsuccessful.

[067] Another set of attempts used the HEK293 human cell line, which has a membrane potential of -30 mV. Initial attempts to achieve transient expression were successful in small scale. The cells expression was variable, but it was possible to
20 perform a calcium assay.

[068] An attempt was made to use commercially available inducible lentivirus cell lines, ChanTest Cat# CT6120 Human NMDA Receptor NR1/NR2a Stable Cell Line(HEK293) and ChanTest Cat# CT6121 Human NMDA Receptor NR1/NR2b Stable Cell Line(HEK293). ChanTest Corp. Garfield Heights, OH 44128, USA. High currents were
25 achieved, but it was not possible to perform a calcium assay due to rapid saturation of the signal.

[069] Next, an attempt was made to accomplish functional expression of NMDAR in HEK293 cells using a baculovirus vector.

30 EXAMPLE IIFunctional expression of NMDAR in HEK293 cells using a baculovirus vector

[070] This method provides a transient, fast, flexible assay, with low expression levels to minimize cytotoxicity. The assay is an unbiased screen for optimal protection compounds.

35 [071] Construction of a BacMam vector. We first constructed a Gateway compatible BacMam vector with a strong CMV promoter by combining baculovirus vector elements from pFastBac1 (available from Thermo Fisher Scientific, Waltham MA USA, Cat #

10360-014) with promoter elements from the Checkmate™ pACT plasmid (available from Promega Corporation, Madison WI USA, Cat # C9341). The pACT vector has a strong cytomegalovirus ("CMV") promoter region which includes the CMV immediate early promoter, plus a chimeric intron comprised of the 5'-donor site from the first intron of the human β -globin gene and the branch and 3'-acceptor site from the intron of an immunoglobulin gene heavy chain variable region. For details, see the Promega Technical Manual TM049. The resultant BacMam vector contained the strong CMV promoter and a mammalian-two-hybrid fusion protein.

[072] We used an Agilent QuikChangeII mutagenesis kit (available from Agilent Technologies, Santa Clara CA USA, Cat # 200523) to make the vector. A PCR-fragment containing the CMV promoter, plus the chimeric intron region, plus the ACT-protein fusion from the Checkmate™ plasmid, was used as a long mutagenic primer to introduce the CMV promoter and fusion protein into a pFastBac1 vector (available from Thermo Fisher Scientific, Waltham MA USA). Our use of the primers resulted in deletion of the baculovirus-specific polyhedrin promoter from pFastBac1. Thus, expression from this recombinant vector does not occur in baculovirus particles or in insect cells, but will occur after transduction of permissive mammalian cell types with the BacMam baculovirus vectors produced by insect cell transfection and infection.

[073] This strong promoter BacMam vector was Gateway-adapted by cloning a Gateway® cassette into an XhoI site of the vector. See, the XhoI site in FIG. 4. This removed the pACT-protein fusion from the pFastbac vector described above and added a cassette containing the *attR* recombination sites flanking a *ccdB* gene plus a chloramphenicol-resistance gene. The baculovirus vector was propagated using *ccdB* Survival 2 competent cells (available from Thermo Fisher Scientific, Waltham MA USA, Cat # A10460).

[074] This final baculovirus vector, named "pFastBac1-CMV/GW-DEST", is the parent vector for the NMDAR and Kir2.1 BacMam expression vectors used in these EXAMPLES. A vector map of the pFastBac1-CMV/GW-DEST vector is shown in FIG. 4.

[075] Cloning of NMDAR subunits and Kir2.1 using Gateway reactions. cDNAs for the GRIN1, GRIN2A, GRIN2B and Kir2.1 proteins were either obtained from our in-house clone collection or purchased from GeneCopoeia, Rockville MD USA. The source and accession number for the proteins encoded in the various vectors is shown in TABLE 1 below. The cDNAs from GeneCopoeia were already in the equivalent of Gateway ENTR vectors. To move the GRIN1 or the GRIN2B coding sequences in-frame into our Gateway-compatible vectors, LR reactions were used. For the two cDNAs obtained in pDEST40 vector, we needed an additional Gateway reaction between pENTR221 and the pDEST40 vector using BP Clonase (available from Thermo Fisher Scientific, Waltham MA USA) to first generate ENTR vectors.

TABLE 1 Source Information for cDNA					
cDNA	Alias	Clone source	Accession #	Original vector	Vendor Cat. #
GRIN1	NR1	GeneCopoeia	NM_007327	EZShuttle™ Gateway® PLUS ORF clones	HOC23110
GRIN2A	NR2A	GeneCopoeia	NM_000833.3	pDEST40	GC-A1441
GRIN2B	NR2B		NM_000834.3	EZShuttle™ Gateway® PLUS ORF clones	
KCNJ2	Kir2.1		NM_000891.2	pDEST40	

[076] BacMam virus production in *E. coli* and insect cells. For the production of recombinant baculovirus vectors (bacmids), we followed the directions in the Invitrogen pFastBac manual. See, Thermo Fisher Scientific, Waltham MA USA. In this system, recombination occurs in a permissive *E. coli* strain, DH10Bac™, between two Tn7 recombination sites in the expression plasmid and a Tn7 transposase mini-attTn7 element present in a bacmid in the DH10Bac™ *E. coli* cells. As described above, this bacmid is a baculovirus shuttle vector containing the majority of the baculovirus genome. After transformation of DH10Bac™ cells, recombinant bacmid are recognized by a blue-white color change.

[077] Bacmid DNA is prepared from white colonies and is used to transform insect cells. Approximately 5-6 days after transformation, the insect cells lyse due to their infection with baculoviruses. The cell supernatants which contain the P1 viruses were filtered and added to 150 ml of Sf9 (*Spodoptera frugiperda*) insect cells at 2×10^6 /ml in shaker flasks, and incubated in an INFORS shaking incubator at 27°C at 100 rpm for 5-6 days to generate P2 viruses. P3 viruses were generated using P2 viruses using a 1:100 (v/v) dilution of the virus-containing supernatant to insect cells at 2×10^6 /ml.

[078] Titratable expression of NMDAR protein in mammalian cells. We then used baculovirus vector-mediated expression of NMDAR subunits in mammalian cells to achieve a rapid, titratable expression of NMDAR protein in cells. See, FIG. 1.

[079] Transduction of HEK293 with different amounts of baculovirus vectors encoding GRIN1 (NR1) or GRIN2A (NR2A) in the presence of 1 mM ketamine caused the expression of both NMDAR subunits after only sixteen hours, as assessed by immunoblotting. See, FIG. 1. For immunoblotting techniques, see Green & Sambrook,

5 *Molecular Cloning: A Laboratory Manual, Fourth Edition* (Cold Spring Harbor Press, Plainview, NY, 2012).

[080] The NMDAR expression levels correlated with the amount of virus added, demonstrating the advantage of now making it possible neuroscientists and biochemists to titrate the expression level of NMDAR by adjusting the amount of virus. We found that
10 the baculovirus-expressed NMDAR protein was functional, as demonstrated by manual patch clamp measurements of NMDAR-dependent currents in the baculovirus-transduced HEK293 cells.

[081] Titration experiments showed that cell viability, and hence the expression of functional NMDAR in the cells, was dependent upon the expression levels of both the
15 NR1 and NR2A subunits. TABLE 2 shows the cell viability of HEK293 cells sixteen hours after transduction with different amounts of baculovirus vector encoding for NR1 or NR2A. Cell viability was assessed using CellTiter-Glo®. Data were normalized to non-transduced cells and represent the mean $\pm$ standard deviation (STDEV), n=3.

TABLE 2				
Raw Data Viability (Cell Titer GloTec®)				
	NR2A (MOI 0) (0 μl)			
NR2A (MOI 0) (0 μ l)	7536	9600	7540	9172
NR2A (MOI 1000) (2 μ l)	10272	10264	8764	10100
NR2A (MOI 375) (0.75 μ l)	10628	11380	10256	10624
NR2A (MOI 125) (0.25 μ l)	11172	11136	10488	9888
	NR2A (MOI 1000) (2 μl)			
NR2A (MOI 0) (0 μ l)	10988	8852	10548	11208
NR2A (MOI 1000) (2 μ l)	1108	1056	1072	1112
NR2A (MOI 375) (0.75 μ l)	3448	3468	2896	3420
NR2A (MOI 125) (0.25 μ l)	8700	7144	6336	7868
	NR2A (MOI 375) (0.75 μl)			
NR2A (MOI 0) (0 μ l)	10160	9552	11184	9812
NR2A (MOI 1000) (2 μ l)	1856	2092	2248	1952
NR2A (MOI 375) (0.75 μ l)	3752	3532	2892	2820
NR2A (MOI 125) (0.25 μ l)	5804	6508	6236	5644
	NR2A (MOI 125) (0.25 μl)			
NR2A (MOI 0) (0 μ l)	10240	10056	9824	10240
NR2A (MOI 1000) (2 μ l)	5600	5376	3556	3860
NR2A (MOI 375) (0.75 μ l)	5452	5448	5236	5648
NR2A (MOI 125) (0.25 μ l)	6624	6832	6992	6908

20 **[082]** The data in TABLE 2 showed the ability to titrate the expression of each subunit to obtain optimal functional expression of NMDAR, thus corroborating the advantages of baculovirus-mediated expression of NMDAR.

EXAMPLE III

The glycine binding site antagonist MDL105,519 - but not ketamine or MK801 – protects cells from cytotoxicity and facilitates the measurement of NMDAR activity in a functional assay

[083] The NMDAR-mediated cytotoxicity observed after transduction of HEK293 cells with baculovirus encoding NR1/2A was rescued by addition of the channel blockers ketamine and MK801 in a concentration dependent manner.

[084] We transduced HEK293 cells with NR1/2A baculovirus vector in the presence of MDL105,519 and loaded the cells with calcium 6 dye. We measured the NMDAR-mediated calcium flux after stimulation of both ketamine and MK801 at the concentrations indicated in TABLE 3.

[085] The measurement of the ligand concentration dependency of NMDAR-mediated calcium flux (as a fluorescence ratio) is by the same procedure as for TABLE 2. We measured the viability of HEK293 cells sixteen hours after transduction with the baculovirus vector encoding for NR1 or NR2A and concurrent treatment with MK801 or ketamine. The cell viability was assessed using CellTiter-Glo®. The data were normalized to non-transduced cells in the presence of solvent. Each datum represents the mean $\pm$ standard deviation of a representative experiment.

TABLE 3
Relative Cell Viability vs. NMDAR Channel Blocker Concentration

<u>MK801</u> concentration (μ M)	<u>no virus</u>		<u>MOI 1000</u> (2+2 μ l virus)		<u>MOI 250</u> (0.5+0.5 μ l virus)	
100.00	0.94	0.88	0.87	0.69	0.93	0.93
50.00	0.95	0.97	0.81	0.59	0.90	0.96
25.00	0.96	0.96	0.67	0.55	0.80	0.85
12.50	0.99	0.91	0.58	0.48	0.80	0.76
6.00	1.01	0.96	0.53	0.45	0.76	0.71
3.00	0.99	0.96	0.48	0.37	0.72	0.73
1.50	1.03	0.94	0.42	0.33	0.55	0.56
0.75	1.02	1.00	0.36	0.33	0.57	0.53
<u>Ketamine</u> concentration (μ M)	<u>no virus</u>		<u>MOI 1000</u> (2+2 μ l virus)		<u>MOI 250</u> (0.5+0.5 μ l virus)	
5.00	0.13	0.11	0.29	0.32	0.12	0.16
2.50	0.54	0.54	0.78	0.81	0.61	0.70
1.25	0.84	0.83	0.62	0.55	0.77	0.77
0.60	0.88	0.82	0.43	0.42	0.62	0.61
0.30	0.95	0.93	0.31	0.35	0.57	0.54
0.15	0.94	0.90	0.28	0.29	0.51	0.53
0.08	0.94	0.97	0.24	0.27	0.44	0.44
0.04	1.02	0.96	0.25	0.27	0.43	0.48

The concentrations and data were rounded to two decimal places.

- [086]** Cytotoxicity is manageable with NMDAR inhibitors. We tested whether ketamine-mediated or MK801-mediated protection of the cells from excitotoxicity would also enable us to measure NMDAR activity in a calcium flux assay. HEK293 cells were transduced with two different amounts of baculovirus vectors encoding for NR1/2A and treated with
- 5 channel blockers (ketamine or MK801) at the indicated concentrations. Sixteen hours after transduction, the cells were washed several times with assay buffer to remove the inhibitors and loaded with calcium 6 dye. NMDAR activity was measured on a fluorescent plate reader (FDSS) after addition of 100 μ M glycine and glutamate. Cell viability was assessed by CellTiter-Glo[®] after the measurement.
- 10 **[087]** Ketamine and MK801 are NMDAR channel blockers that prevented NMDAR-mediated excitotoxicity. We were not able to measure NMDAR-mediated calcium influx in the cells even after multiple washes of the cells. Thus, ketamine-mediated and MK801-mediated protections from excitotoxicity are not suitable for the measurement of NMDAR activity in a microplate based assay, likely because of the inability to completely wash out
- 15 the inhibitors. See, FIG. 2 "Protection with channel blocker (Current state of the art)".
- [088]** We then tested whether an allosteric negative modulator (TCN201), an NMDAR ligand site binding antagonist (MDL105,519), AP5 or $MgCl_2$ would be easier to wash out than the NMDAR channel blockers and would enable us to measure NMDAR activity. HEK293 cells were transduced as described above and treated with the indicated
- 20 inhibitors. We measured the cell viability of HEK293 cells sixteen hours after transduction with baculovirus encoding for NR1 or NR2A and concurrent treatment with the indicated NMDAR-inhibitors. Cell viability was measured using CellTiter-Glo[®]. The data were normalized to non-transduced cells in the presence of solvent and represents the mean $\pm$ standard deviation (STDEV) of a representative experiment.

[089] The data in TABLE 4 shows that AP5 and TCN201 were not sufficient to protect the cells from excitotoxicity. MgCl₂ and MDL105,519 showed partial rescue.

TABLE 4 Relative Cell Viability							
AP5 concentration μ M	no virus		MOI 1000 (2+2 μ l virus)		MOI 250 (0.5+0.5 μ l virus)		
100.00	0.94	1.03	0.23	0.25	0.45	0.51	
50.00	1.01	1.00	0.23	0.23	0.47	0.44	
25.00	1.00	0.99	0.24	0.25	0.49	0.50	
12.50	1.00	1.01	0.27	0.26	0.48	0.49	
6.00	0.98	1.00	0.28	0.30	0.54	0.51	
3.00	0.95	1.00	0.26	0.26	0.42	0.42	
1.50	1.01	1.06	0.26	0.27	0.43	0.39	
0.75	0.98	0.99	0.25	0.23	0.44	0.48	
TCN201 concentration μ M	no virus		MOI 1000 (2+2 μ l virus)		MOI 250 (0.5+0.5 μ l virus)		
100.00	0.63	0.60	0.24	0.25	0.42	0.42	
50.00	0.79	0.77	0.29	0.29	0.48	0.51	
25.00	0.98	0.95	0.27	0.29	0.45	0.47	
12.50	0.98	0.95	0.26	0.26	0.45	0.49	
6.00	1.02	1.03	0.27	0.23	0.45	0.57	
3.00	1.03	0.96	0.26	0.27	0.43	0.45	
1.50	1.03	1.01	0.26	0.28	0.38	0.45	
0.75	1.03	0.96	0.26	0.28	0.51	0.50	
MDL105,519 concentration μ M	no virus		MOI 1000 (2+2 μ l virus)		MOI 250 (0.5+0.5 μ l virus)		
100.00	1.04	1.10	0.43	0.39	0.74	0.75	
50.00	1.04	1.10	0.41	0.38	0.71	0.63	
25.00	1.05	1.09	0.32	0.32	0.63	0.65	
12.50	0.92	1.07	0.31	0.30	0.58	0.55	
6.00	0.99	1.00	0.27	0.27	0.53	0.55	
3.00	0.98	0.98	0.29	0.25	0.49	0.51	
1.50	1.01	1.02	0.27	0.24	0.52	0.46	
0.75	0.92	1.01	0.24	0.25	0.46	0.50	
MgCl ₂ concentration mM	no virus		MOI 1000 (2+2 μ l virus)		MOI 250 (0.5+0.5 μ l virus)		
100.00	0.37	0.39	0.48	0.57	0.28	0.29	
50.00	0.70	0.74	0.65	0.64	0.69	0.80	
25.00	0.95	0.94	0.42	0.40	0.74	0.74	
12.50	0.98	1.03	0.29	0.31	0.55	0.62	
6.00	1.04	1.00	0.24	0.24	0.45	0.49	
3.00	1.00	1.02	0.26	0.26	0.47	0.45	
1.50	1.01	0.99	0.27	0.24	0.43	0.47	
0.75	1.02	1.02	0.25	0.29	0.43	0.50	

Concentrations and data were rounded to two decimal places

- [090]** NMDAR-mediated calcium flux in HEK293 cells transduced with baculovirus vectors. Despite the only 50% rescue in cell viability, protection of the cells with MDL105,519 followed by wash out of the compound resulted in a significant and stable signal in our calcium flux assay. None of the other inhibitors showed significant NMDAR activity.

- [091] HEK293 cells were transduced with NMDAR-encoding baculovirus vectors as described above and loaded with calcium 6 dye. NMDAR-mediated calcium flux was measured after stimulation with 100 μM glycine/glutamate. Data shown in TABLE 5 represent the mean $\pm$ STDEV of the maximal fluorescence ratio (maximal fluorescence/baseline fluorescence, F_{max}/F_0) of a representative experiment. Units are mM for MgCl_2 and μM for all other inhibitors.

TABLE 5			
Protection of Transfected Cells by MDL105,519			
(Compound F_{max}/F_0)			
<u>MgCl₂</u>			
100.00 $\mu\text{M}/\text{mM}$	1.01	1.01	
50.00 $\mu\text{M}/\text{mM}$	1.05	1.06	
25.00 $\mu\text{M}/\text{mM}$	1.05	1.05	
12.50 $\mu\text{M}/\text{mM}$	1.02	1.03	
6.25 $\mu\text{M}/\text{mM}$	1.02	1.01	
3.13 $\mu\text{M}/\text{mM}$	1.01	1.01	
1.56 $\mu\text{M}/\text{mM}$	1.00	1.00	
0.78 $\mu\text{M}/\text{mM}$	1.01	1.00	
<u>MDL105,519</u>			
100.00 $\mu\text{M}/\text{mM}$	1.89	1.80	
50.00 $\mu\text{M}/\text{mM}$	1.55	1.56	
25.00 $\mu\text{M}/\text{mM}$	1.44	1.39	
12.50 $\mu\text{M}/\text{mM}$	1.19	1.18	
6.25 $\mu\text{M}/\text{mM}$	1.11	1.14	
3.13 $\mu\text{M}/\text{mM}$	1.06	1.08	
1.56 $\mu\text{M}/\text{mM}$	1.04	1.09	
0.78 $\mu\text{M}/\text{mM}$	1.04	1.04	
<u>MK801</u>			
100.00 $\mu\text{M}/\text{mM}$	1.22	1.24	
50.00 $\mu\text{M}/\text{mM}$	1.26	1.21	
25.00 $\mu\text{M}/\text{mM}$	1.23	1.23	
12.50 $\mu\text{M}/\text{mM}$	1.21	1.23	
6.25 $\mu\text{M}/\text{mM}$	1.18	1.16	
3.13 $\mu\text{M}/\text{mM}$	1.16	1.18	
1.56 $\mu\text{M}/\text{mM}$	1.10	1.09	
0.78 $\mu\text{M}/\text{mM}$	1.06	1.07	
<u>Ketamine</u>			
100.00 $\mu\text{M}/\text{mM}$	1.01	1.00	
50.00 $\mu\text{M}/\text{mM}$	1.06	1.05	
25.00 $\mu\text{M}/\text{mM}$	1.13	1.17	
12.50 $\mu\text{M}/\text{mM}$	1.1	1.09	
6.25 $\mu\text{M}/\text{mM}$	1.07	1.07	
3.13 $\mu\text{M}/\text{mM}$	1.05	1.04	
1.56 $\mu\text{M}/\text{mM}$	1.02	1.01	
0.78 $\mu\text{M}/\text{mM}$	1.01	1	
<u>AP5</u>			
100.00 $\mu\text{M}/\text{mM}$	1.03	1.03	
50.00 $\mu\text{M}/\text{mM}$	1.01	1.04	
25.00 $\mu\text{M}/\text{mM}$	1.02	1.02	
12.50 $\mu\text{M}/\text{mM}$	1.01	1	
6.25 $\mu\text{M}/\text{mM}$	1.03	1.01	
3.13 $\mu\text{M}/\text{mM}$	1.01	1	
1.56 $\mu\text{M}/\text{mM}$	1.02	1.02	
0.78 $\mu\text{M}/\text{mM}$	1.01	1.02	

TABLE 5 Protection of Transfected Cells by MDL105,519 (Compound $F_{\max}/F_0$)			
TC201			
100.00 $\mu\text{M}/\text{mM}$	1.26	1.31	
50.00 $\mu\text{M}/\text{mM}$	1.28	1.28	
25.00 $\mu\text{M}/\text{mM}$	1.11	1.09	
12.50 $\mu\text{M}/\text{mM}$	1.10	1.08	
6.25 $\mu\text{M}/\text{mM}$	1.04	1.06	
3.13 $\mu\text{M}/\text{mM}$	1.05	1.02	
1.56 $\mu\text{M}/\text{mM}$	1.05	1.04	
0.78 $\mu\text{M}/\text{mM}$	1.05	1.03	
Concentration and data were rounded to two decimal places			

- [092]** Because the activity of NMDAR is expected to be dependent on the expression ratio of the subunits, we optimized the amount of baculovirus needed to transduce the cells to gain optimal functional amounts of NMDAR by titrating both viruses and measuring NMDAR-mediated calcium flux after protection of the cells with MDL105,519.
- [093]** TABLE 6 shows the titration of the maximal NMDAR-mediated calcium flux in HEK293 cells transduced with baculovirus. Cells were transduced with different ratios/amounts of NR1 and NR2A in the presence of MDL105,519 and NMDAR-mediated calcium flux was measured as in (B) after washout of MDL105,519. Data in TABLE 6 represent the maximal fluorescence ratio, $\pm$ STDEV, $n=4$.

TABLE 6 Titration of the Maximal NMDAR-Mediated Calcium Flux in HEK293 Cells Transduced with Baculovirus.						
	NR2A (MOI)					
	0	1000	500	250	125	
NR1 (MOI)	0	1.0	1.0	1.0	1.0	1.0
	1000	1.0	2.5	2.7	2.7	2.6
	500	1.0	2.7	2.8	2.5	2.6
	250	1.0	2.8	2.9	2.7	2.3
	125	1.0	2.6	2.7	2.7	2.3

- [094]** A multiplicity of infection (MOI) ratio of 250xNR1 : 500xNR2A was found to be optimal and was used for all subsequent experiments.
- [095]** Ligand concentration dependency of NMDAR-mediated calcium flux. Stimulation of NR1/2A-transduced and MDL105,519 protected HEK293 cells with varying concentrations of glycine and glutamate showed a ligand-concentration dependent activity of NMDAR as measured by NMDAR-mediated calcium influx. HEK293 cells were transduced with NR1/2A baculovirus in the presence of MDL105,519, loaded with calcium 6 dye and NMDAR-mediated calcium flux was measured after stimulation of the indicated concentration of both ligands. Data in TABLE 7 represent the fluorescence ratio of a representative experiment, rounded to two decimal places.

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
0.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.0	1.00	1.01	1.01	1.00	1.00	0.99	1.00	1.00	0.99
0.5	1.00	1.00	1.00	0.99	1.00	0.98	1.00	0.99	1.00
1.0	0.99	1.00	0.99	1.00	1.00	0.99	0.99	0.98	0.99
1.5	0.98	1.00	0.99	1.00	1.00	0.99	0.99	0.99	0.99
2.0	1.00	1.00	1.00	0.99	1.00	0.98	0.99	1.00	0.99
2.5	0.99	1.00	0.99	0.99	1.00	0.99	0.99	1.00	0.99
3.0	1.00	1.00	1.00	0.99	0.99	0.98	0.99	0.99	1.00
3.5	0.99	1.00	0.99	0.99	1.00	0.98	0.99	0.98	0.99
4.0	0.99	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99
4.5	0.99	0.99	0.99	0.99	1.00	0.99	0.99	0.98	0.99
5.0	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
5.5	0.96	0.98	0.98	0.98	0.98	0.97	0.97	0.97	0.98
6.0	0.98	0.99	1.00	0.99	0.98	0.99	0.98	0.98	0.99
6.5	0.99	1.00	0.99	0.99	0.99	1.00	0.99	0.99	0.99
7.0	0.99	1.00	1.00	1.00	1.00	1.01	0.99	0.98	1.00
7.5	1.00	1.00	1.01	1.00	1.00	1.01	1.00	0.99	1.00
8.0	1.01	1.01	1.02	1.02	1.01	1.02	1.00	1.00	1.00
8.5	1.02	1.02	1.03	1.03	1.01	1.02	1.01	1.00	1.00
9.0	1.02	1.03	1.03	1.03	1.02	1.03	1.01	1.00	1.01
9.5	1.03	1.04	1.04	1.04	1.03	1.04	1.02	1.02	1.01
10.0	1.04	1.04	1.04	1.04	1.04	1.04	1.02	1.02	1.01
10.5	1.04	1.06	1.05	1.04	1.04	1.04	1.03	1.01	1.01
11.0	1.04	1.05	1.05	1.04	1.03	1.04	1.02	1.02	1.01
11.5	1.05	1.05	1.06	1.05	1.04	1.05	1.02	1.02	1.00
12.0	1.07	1.06	1.07	1.06	1.05	1.06	1.03	1.02	1.01
12.5	1.07	1.07	1.08	1.06	1.06	1.06	1.03	1.03	1.01
13.0	1.07	1.08	1.09	1.07	1.06	1.07	1.04	1.03	1.01
13.5	1.08	1.08	1.09	1.08	1.07	1.08	1.04	1.04	1.02
14.0	1.09	1.08	1.10	1.09	1.08	1.08	1.05	1.03	1.01
14.5	1.10	1.10	1.11	1.09	1.08	1.09	1.04	1.03	1.01
15.0	1.11	1.10	1.12	1.10	1.08	1.09	1.05	1.04	1.01
15.5	1.12	1.11	1.13	1.11	1.09	1.11	1.05	1.05	1.02
16.0	1.13	1.13	1.14	1.11	1.10	1.11	1.05	1.04	1.02
16.5	1.14	1.13	1.14	1.12	1.11	1.11	1.07	1.05	1.02
17.0	1.14	1.13	1.15	1.14	1.10	1.11	1.06	1.05	1.01
17.5	1.16	1.14	1.16	1.14	1.11	1.12	1.06	1.05	1.02
18.0	1.16	1.16	1.17	1.14	1.12	1.12	1.07	1.05	1.02
18.5	1.18	1.17	1.17	1.15	1.12	1.13	1.08	1.06	1.01
19.0	1.18	1.18	1.19	1.16	1.12	1.14	1.08	1.06	1.01
19.5	1.19	1.19	1.20	1.17	1.15	1.14	1.08	1.07	1.03
20.0	1.20	1.19	1.20	1.17	1.14	1.14	1.08	1.06	1.02
20.5	1.21	1.20	1.22	1.18	1.15	1.15	1.08	1.07	1.02
21.0	1.21	1.21	1.22	1.18	1.15	1.15	1.08	1.07	1.03
21.5	1.22	1.22	1.23	1.19	1.16	1.16	1.09	1.07	1.03
22.0	1.23	1.22	1.24	1.20	1.15	1.16	1.09	1.06	1.03
22.5	1.24	1.23	1.24	1.20	1.16	1.17	1.09	1.07	1.02
23.0	1.25	1.24	1.26	1.21	1.17	1.17	1.10	1.08	1.03
23.5	1.26	1.25	1.26	1.22	1.18	1.17	1.09	1.07	1.03
24.0	1.28	1.26	1.27	1.22	1.19	1.18	1.10	1.08	1.03
24.5	1.28	1.26	1.29	1.23	1.19	1.18	1.09	1.07	1.03
25.0	1.29	1.27	1.29	1.24	1.19	1.18	1.10	1.08	1.02
25.5	1.30	1.28	1.30	1.25	1.20	1.18	1.11	1.08	1.03

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
26.0	1.32	1.30	1.31	1.25	1.20	1.19	1.10	1.08	1.02
26.5	1.33	1.30	1.32	1.27	1.20	1.20	1.10	1.08	1.03
27.0	1.34	1.32	1.33	1.26	1.21	1.20	1.11	1.08	1.03
27.5	1.34	1.32	1.34	1.27	1.21	1.21	1.11	1.09	1.03
28.0	1.35	1.34	1.35	1.28	1.22	1.20	1.11	1.08	1.03
28.5	1.35	1.34	1.36	1.29	1.23	1.21	1.12	1.08	1.03
29.0	1.38	1.35	1.37	1.30	1.23	1.22	1.12	1.09	1.03
29.5	1.38	1.36	1.39	1.29	1.23	1.21	1.12	1.08	1.03
30.0	1.38	1.38	1.38	1.30	1.24	1.21	1.11	1.09	1.03
30.5	1.40	1.38	1.39	1.31	1.25	1.22	1.12	1.08	1.03
31.0	1.42	1.38	1.39	1.32	1.25	1.22	1.12	1.09	1.03
31.5	1.42	1.39	1.41	1.33	1.25	1.23	1.12	1.10	1.03
32.0	1.43	1.40	1.42	1.33	1.26	1.22	1.12	1.09	1.03
32.5	1.44	1.41	1.42	1.33	1.26	1.23	1.12	1.09	1.03
33.0	1.45	1.42	1.43	1.34	1.26	1.24	1.13	1.10	1.03
33.5	1.46	1.43	1.44	1.34	1.27	1.23	1.13	1.09	1.03
34.0	1.47	1.44	1.45	1.35	1.27	1.24	1.14	1.10	1.03
34.5	1.48	1.44	1.45	1.36	1.28	1.24	1.13	1.09	1.03
35.0	1.49	1.45	1.47	1.36	1.28	1.24	1.14	1.10	1.03
35.5	1.49	1.47	1.47	1.37	1.29	1.26	1.14	1.10	1.03
36.0	1.51	1.47	1.48	1.37	1.28	1.24	1.13	1.10	1.03
36.5	1.52	1.47	1.49	1.38	1.30	1.25	1.14	1.10	1.03
37.0	1.52	1.49	1.50	1.39	1.29	1.25	1.14	1.10	1.03
37.5	1.53	1.50	1.51	1.39	1.30	1.25	1.14	1.10	1.03
38.0	1.54	1.50	1.50	1.39	1.31	1.25	1.14	1.10	1.03
38.5	1.55	1.50	1.51	1.41	1.30	1.26	1.14	1.10	1.03
39.0	1.56	1.52	1.53	1.41	1.31	1.26	1.14	1.10	1.03
39.5	1.56	1.53	1.54	1.42	1.31	1.26	1.14	1.10	1.03
40.0	1.57	1.54	1.53	1.41	1.31	1.26	1.14	1.09	1.03
40.5	1.58	1.55	1.55	1.42	1.33	1.26	1.14	1.10	1.04
41.0	1.59	1.55	1.55	1.43	1.33	1.26	1.14	1.09	1.04
41.5	1.60	1.57	1.57	1.44	1.33	1.26	1.14	1.10	1.03
42.0	1.61	1.56	1.57	1.44	1.33	1.27	1.15	1.10	1.03
42.5	1.62	1.57	1.58	1.44	1.35	1.26	1.15	1.11	1.03
43.0	1.63	1.58	1.58	1.45	1.33	1.28	1.15	1.09	1.03
43.5	1.63	1.59	1.59	1.46	1.34	1.28	1.14	1.10	1.04
44.0	1.64	1.59	1.60	1.46	1.34	1.28	1.15	1.10	1.03
44.5	1.65	1.61	1.61	1.47	1.34	1.27	1.15	1.11	1.03
45.0	1.66	1.61	1.61	1.46	1.34	1.27	1.14	1.10	1.03
45.5	1.66	1.62	1.61	1.47	1.35	1.28	1.15	1.10	1.04
46.0	1.67	1.63	1.62	1.47	1.36	1.28	1.15	1.11	1.03
46.5	1.69	1.62	1.63	1.48	1.36	1.28	1.15	1.10	1.03
47.0	1.68	1.64	1.64	1.48	1.36	1.28	1.15	1.10	1.03
47.5	1.69	1.64	1.63	1.48	1.35	1.28	1.14	1.10	1.03
48.0	1.70	1.65	1.65	1.49	1.36	1.28	1.15	1.10	1.04
48.5	1.70	1.66	1.65	1.49	1.36	1.28	1.15	1.10	1.03
49.0	1.71	1.66	1.66	1.49	1.36	1.28	1.16	1.10	1.04
49.5	1.72	1.66	1.66	1.50	1.37	1.28	1.15	1.10	1.03
50.0	1.72	1.68	1.67	1.50	1.37	1.28	1.15	1.10	1.03
50.5	1.73	1.68	1.68	1.51	1.37	1.29	1.16	1.11	1.04
51.0	1.73	1.69	1.67	1.51	1.38	1.29	1.15	1.10	1.03
51.5	1.76	1.70	1.69	1.52	1.38	1.30	1.15	1.11	1.03
52.0	1.75	1.70	1.69	1.52	1.37	1.30	1.15	1.10	1.03

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
52.5	1.77	1.70	1.71	1.53	1.39	1.29	1.15	1.10	1.03
53.0	1.76	1.71	1.69	1.52	1.38	1.29	1.15	1.09	1.03
53.5	1.77	1.73	1.71	1.53	1.38	1.29	1.16	1.10	1.03
54.0	1.78	1.73	1.72	1.54	1.39	1.30	1.16	1.10	1.03
54.5	1.78	1.73	1.72	1.53	1.39	1.29	1.15	1.10	1.04
55.0	1.79	1.74	1.73	1.55	1.39	1.29	1.16	1.10	1.03
55.5	1.79	1.75	1.72	1.55	1.39	1.30	1.16	1.10	1.03
56.0	1.80	1.75	1.72	1.55	1.39	1.30	1.15	1.10	1.04
56.5	1.81	1.76	1.73	1.54	1.39	1.29	1.16	1.09	1.03
57.0	1.82	1.76	1.73	1.56	1.40	1.30	1.16	1.10	1.03
57.5	1.82	1.77	1.75	1.56	1.40	1.30	1.16	1.10	1.03
58.0	1.82	1.77	1.74	1.56	1.41	1.30	1.15	1.10	1.03
58.5	1.82	1.77	1.75	1.57	1.41	1.30	1.15	1.10	1.03
59.0	1.84	1.78	1.75	1.57	1.41	1.30	1.15	1.10	1.03
59.5	1.84	1.79	1.76	1.57	1.41	1.31	1.15	1.09	1.03
60.0	1.86	1.80	1.77	1.58	1.41	1.30	1.16	1.09	1.03
60.5	1.86	1.79	1.76	1.58	1.40	1.30	1.16	1.10	1.03
61.0	1.85	1.80	1.78	1.58	1.41	1.30	1.16	1.09	1.03
61.5	1.85	1.81	1.78	1.57	1.41	1.30	1.16	1.10	1.03
62.0	1.87	1.82	1.78	1.59	1.41	1.31	1.16	1.09	1.03
62.5	1.86	1.82	1.78	1.58	1.41	1.30	1.16	1.10	1.03
63.0	1.88	1.83	1.79	1.60	1.42	1.30	1.17	1.10	1.03
63.5	1.88	1.84	1.79	1.59	1.42	1.30	1.15	1.09	1.03
64.0	1.89	1.83	1.80	1.60	1.42	1.29	1.15	1.10	1.03
64.5	1.88	1.83	1.80	1.59	1.42	1.30	1.16	1.10	1.03
65.0	1.89	1.85	1.81	1.61	1.42	1.30	1.16	1.10	1.03
65.5	1.91	1.85	1.81	1.61	1.43	1.30	1.15	1.09	1.03
66.0	1.89	1.85	1.81	1.61	1.43	1.30	1.16	1.09	1.03
66.5	1.91	1.86	1.80	1.61	1.43	1.31	1.16	1.10	1.03
67.0	1.92	1.87	1.83	1.61	1.43	1.31	1.16	1.10	1.03
67.5	1.92	1.88	1.83	1.62	1.43	1.31	1.16	1.09	1.03
68.0	1.92	1.87	1.82	1.62	1.43	1.30	1.16	1.09	1.02
68.5	1.93	1.87	1.83	1.62	1.44	1.30	1.16	1.10	1.03
69.0	1.93	1.87	1.82	1.62	1.44	1.30	1.15	1.09	1.03
69.5	1.93	1.88	1.85	1.62	1.45	1.30	1.16	1.10	1.04
70.0	1.94	1.89	1.84	1.62	1.43	1.30	1.15	1.09	1.03
70.5	1.95	1.89	1.85	1.63	1.44	1.30	1.16	1.10	1.03
71.0	1.93	1.91	1.85	1.63	1.43	1.30	1.15	1.09	1.03
71.5	1.95	1.91	1.85	1.63	1.44	1.30	1.15	1.09	1.03
72.0	1.95	1.89	1.85	1.63	1.44	1.30	1.15	1.09	1.02
72.5	1.96	1.91	1.85	1.64	1.44	1.31	1.16	1.09	1.03
73.0	1.96	1.91	1.86	1.63	1.45	1.31	1.15	1.09	1.03
73.5	1.97	1.92	1.87	1.64	1.44	1.31	1.15	1.09	1.02
74.0	1.96	1.92	1.86	1.64	1.44	1.30	1.15	1.09	1.02
74.5	1.98	1.93	1.86	1.65	1.44	1.30	1.16	1.09	1.02
75.0	1.98	1.92	1.87	1.64	1.45	1.30	1.15	1.09	1.04
75.5	1.98	1.93	1.87	1.64	1.44	1.30	1.16	1.08	1.02
76.0	1.99	1.94	1.88	1.65	1.45	1.30	1.15	1.09	1.03
76.5	1.99	1.94	1.88	1.65	1.44	1.29	1.14	1.09	1.02
77.0	1.98	1.94	1.88	1.65	1.45	1.30	1.15	1.08	1.02
77.5	2.00	1.95	1.89	1.66	1.46	1.30	1.15	1.08	1.03
78.0	1.99	1.96	1.88	1.65	1.45	1.30	1.15	1.09	1.03
78.5	2.01	1.95	1.89	1.66	1.45	1.31	1.16	1.09	1.02

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
79.0	2.01	1.96	1.89	1.66	1.44	1.30	1.15	1.08	1.03
79.5	2.01	1.96	1.89	1.66	1.45	1.30	1.15	1.08	1.02
80.0	2.01	1.97	1.91	1.66	1.45	1.29	1.16	1.08	1.03
80.5	2.01	1.97	1.90	1.67	1.46	1.30	1.15	1.08	1.03
81.0	2.02	1.97	1.90	1.66	1.46	1.30	1.15	1.09	1.03
81.5	2.03	1.98	1.90	1.68	1.45	1.30	1.15	1.08	1.02
82.0	2.03	1.98	1.91	1.66	1.45	1.29	1.15	1.08	1.02
82.5	2.03	1.98	1.91	1.67	1.46	1.30	1.15	1.08	1.03
83.0	2.03	1.97	1.90	1.67	1.45	1.29	1.14	1.08	1.03
83.5	2.04	1.99	1.92	1.67	1.45	1.29	1.15	1.07	1.02
84.0	2.04	1.99	1.91	1.67	1.45	1.29	1.14	1.08	1.02
84.5	2.04	1.99	1.92	1.68	1.45	1.30	1.15	1.08	1.03
85.0	2.06	2.01	1.93	1.68	1.46	1.30	1.15	1.08	1.03
85.5	2.05	1.99	1.92	1.68	1.45	1.30	1.15	1.08	1.02
86.0	2.05	2.00	1.93	1.68	1.46	1.30	1.15	1.08	1.02
86.5	2.05	2.00	1.92	1.67	1.45	1.29	1.14	1.07	1.02
87.0	2.06	2.01	1.92	1.68	1.46	1.29	1.14	1.07	1.02
87.5	2.06	2.02	1.92	1.68	1.46	1.29	1.15	1.06	1.02
88.0	2.08	2.01	1.94	1.69	1.46	1.29	1.14	1.07	1.02
88.5	2.07	2.02	1.95	1.69	1.46	1.29	1.14	1.07	1.02
89.0	2.07	2.02	1.93	1.69	1.46	1.30	1.14	1.07	1.02
89.5	2.06	2.03	1.94	1.69	1.46	1.29	1.14	1.07	1.02
90.0	2.07	2.03	1.94	1.69	1.45	1.29	1.14	1.07	1.02
90.5	2.07	2.03	1.95	1.69	1.46	1.28	1.14	1.07	1.02
91.0	2.09	2.04	1.95	1.70	1.46	1.29	1.15	1.07	1.02
91.5	2.08	2.04	1.95	1.70	1.46	1.28	1.14	1.07	1.02
92.0	2.08	2.04	1.97	1.70	1.46	1.29	1.14	1.07	1.02
92.5	2.09	2.05	1.95	1.70	1.46	1.29	1.14	1.07	1.02
93.0	2.09	2.05	1.95	1.70	1.47	1.29	1.14	1.06	1.02
93.5	2.08	2.04	1.96	1.70	1.46	1.28	1.14	1.06	1.02
94.0	2.09	2.04	1.96	1.70	1.46	1.28	1.14	1.06	1.02
94.5	2.09	2.05	1.96	1.70	1.47	1.30	1.13	1.07	1.02
95.0	2.10	2.05	1.96	1.70	1.46	1.28	1.14	1.06	1.02
95.5	2.10	2.05	1.96	1.70	1.46	1.28	1.14	1.07	1.02
96.0	2.11	2.06	1.96	1.71	1.46	1.29	1.13	1.07	1.02
96.5	2.10	2.05	1.96	1.71	1.47	1.28	1.13	1.07	1.02
97.0	2.11	2.06	1.96	1.70	1.46	1.28	1.13	1.07	1.01
97.5	2.11	2.07	1.96	1.70	1.47	1.28	1.14	1.07	1.02
98.0	2.12	2.06	1.97	1.71	1.47	1.28	1.14	1.06	1.01
98.5	2.10	2.07	1.98	1.71	1.46	1.28	1.14	1.06	1.01
99.0	2.13	2.07	1.97	1.71	1.46	1.28	1.14	1.06	1.02
99.5	2.12	2.07	1.97	1.71	1.46	1.28	1.13	1.06	1.02
100.0	2.12	2.07	1.98	1.71	1.47	1.28	1.13	1.06	1.02
100.5	2.13	2.08	1.99	1.71	1.47	1.28	1.14	1.07	1.02
101.0	2.12	2.09	1.99	1.71	1.47	1.28	1.14	1.06	1.01
101.5	2.12	2.08	1.98	1.71	1.47	1.28	1.13	1.06	1.02
102.0	2.14	2.09	1.99	1.72	1.47	1.28	1.13	1.06	1.02
102.5	2.14	2.10	2.00	1.72	1.47	1.28	1.14	1.06	1.02
103.0	2.14	2.09	1.99	1.71	1.46	1.28	1.13	1.06	1.01
103.5	2.16	2.09	2.00	1.72	1.47	1.27	1.13	1.06	1.01
104.0	2.16	2.11	2.00	1.73	1.48	1.28	1.14	1.07	1.03
104.5	2.16	2.10	2.00	1.72	1.48	1.28	1.13	1.06	1.02
105.0	2.15	2.11	2.00	1.73	1.47	1.28	1.13	1.06	1.02

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
105.5	2.15	2.10	1.99	1.73	1.47	1.27	1.13	1.06	1.02
106.0	2.16	2.10	2.00	1.73	1.48	1.27	1.14	1.06	1.02
106.5	2.15	2.11	1.99	1.72	1.48	1.28	1.13	1.05	1.02
107.0	2.15	2.11	2.00	1.72	1.47	1.27	1.13	1.06	1.02
107.5	2.16	2.11	2.01	1.72	1.47	1.27	1.13	1.06	1.01
108.0	2.16	2.12	2.01	1.73	1.47	1.27	1.14	1.06	1.01
108.5	2.15	2.12	2.00	1.72	1.47	1.28	1.13	1.06	1.02
109.0	2.16	2.13	2.01	1.73	1.47	1.28	1.13	1.06	1.02
109.5	2.18	2.13	2.01	1.73	1.48	1.28	1.13	1.05	1.02
110.0	2.17	2.13	2.02	1.73	1.47	1.28	1.13	1.06	1.01
110.5	2.17	2.12	2.00	1.73	1.47	1.27	1.13	1.05	1.02
111.0	2.17	2.12	2.01	1.73	1.47	1.26	1.12	1.06	1.01
111.5	2.16	2.14	2.01	1.72	1.47	1.26	1.13	1.05	1.01
112.0	2.17	2.13	2.01	1.72	1.47	1.27	1.13	1.05	1.01
112.5	2.18	2.13	2.02	1.73	1.47	1.27	1.12	1.04	1.02
113.0	2.18	2.13	2.02	1.73	1.47	1.27	1.13	1.05	1.01
113.5	2.18	2.13	2.01	1.73	1.47	1.27	1.12	1.05	1.01
114.0	2.19	2.14	2.02	1.74	1.47	1.27	1.12	1.06	1.02
114.5	2.18	2.13	2.02	1.72	1.47	1.26	1.11	1.04	1.01
115.0	2.19	2.14	2.02	1.73	1.48	1.27	1.12	1.05	1.01
115.5	2.19	2.13	2.01	1.72	1.46	1.26	1.12	1.05	1.01
116.0	2.19	2.15	2.02	1.73	1.47	1.26	1.12	1.04	1.01
116.5	2.19	2.14	2.02	1.73	1.48	1.26	1.13	1.04	1.01
117.0	2.19	2.15	2.02	1.73	1.47	1.26	1.12	1.05	1.01
117.5	2.19	2.15	2.03	1.73	1.46	1.26	1.12	1.05	1.01
118.0	2.20	2.15	2.03	1.74	1.47	1.26	1.11	1.04	1.01
118.5	2.20	2.15	2.02	1.74	1.47	1.26	1.12	1.05	1.01
119.0	2.20	2.15	2.03	1.73	1.47	1.26	1.13	1.05	1.01
119.5	2.19	2.16	2.03	1.73	1.47	1.25	1.12	1.05	1.01
120.0	2.21	2.15	2.03	1.74	1.48	1.26	1.12	1.05	1.01
120.5	2.20	2.15	2.03	1.74	1.46	1.25	1.12	1.04	1.01
121.0	2.20	2.16	2.03	1.73	1.47	1.25	1.12	1.04	1.01
121.5	2.22	2.16	2.04	1.74	1.48	1.26	1.12	1.05	1.01
122.0	2.21	2.17	2.04	1.74	1.47	1.25	1.11	1.04	1.01
122.5	2.22	2.17	2.04	1.73	1.47	1.26	1.11	1.05	1.01
123.0	2.21	2.16	2.04	1.73	1.47	1.26	1.11	1.04	1.01
123.5	2.20	2.16	2.04	1.73	1.47	1.26	1.11	1.04	1.01
124.0	2.21	2.17	2.04	1.74	1.47	1.26	1.11	1.04	1.01
124.5	2.21	2.17	2.04	1.74	1.47	1.25	1.11	1.04	1.01
125.0	2.22	2.18	2.03	1.74	1.46	1.25	1.11	1.03	1.01
125.5	2.22	2.17	2.04	1.74	1.47	1.25	1.11	1.04	1.02
126.0	2.22	2.17	2.03	1.74	1.46	1.25	1.12	1.04	1.01
126.5	2.21	2.17	2.04	1.75	1.47	1.26	1.12	1.03	1.01
127.0	2.22	2.18	2.04	1.74	1.46	1.25	1.11	1.04	1.01
127.5	2.24	2.18	2.06	1.75	1.47	1.25	1.11	1.04	1.01
128.0	2.23	2.18	2.04	1.73	1.47	1.25	1.11	1.04	1.01
128.5	2.22	2.18	2.04	1.73	1.46	1.25	1.11	1.03	1.01
129.0	2.24	2.18	2.05	1.74	1.47	1.25	1.11	1.04	1.01
129.5	2.23	2.19	2.04	1.74	1.47	1.24	1.11	1.04	1.01
130.0	2.24	2.19	2.04	1.74	1.46	1.25	1.11	1.04	1.01
130.5	2.23	2.18	2.06	1.74	1.48	1.25	1.10	1.04	1.01
131.0	2.22	2.18	2.04	1.73	1.47	1.25	1.11	1.04	1.01
131.5	2.22	2.19	2.06	1.74	1.48	1.24	1.11	1.03	1.01

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
132.0	2.24	2.19	2.05	1.74	1.47	1.25	1.10	1.03	1.02
132.5	2.24	2.19	2.06	1.74	1.47	1.24	1.11	1.04	1.01
133.0	2.24	2.19	2.05	1.75	1.47	1.25	1.11	1.04	1.01
133.5	2.24	2.19	2.04	1.74	1.46	1.25	1.11	1.03	1.00
134.0	2.25	2.20	2.04	1.74	1.47	1.24	1.11	1.04	1.01
134.5	2.24	2.20	2.05	1.74	1.47	1.25	1.10	1.03	1.01
135.0	2.24	2.20	2.05	1.73	1.46	1.24	1.10	1.03	1.01
135.5	2.25	2.20	2.06	1.74	1.47	1.24	1.11	1.03	1.01
136.0	2.24	2.20	2.05	1.74	1.46	1.24	1.10	1.02	1.01
136.5	2.25	2.20	2.05	1.75	1.46	1.24	1.10	1.03	1.01
137.0	2.25	2.20	2.05	1.74	1.46	1.23	1.10	1.02	1.00
137.5	2.24	2.20	2.05	1.75	1.46	1.24	1.10	1.03	1.01
138.0	2.26	2.20	2.06	1.75	1.46	1.23	1.10	1.03	1.01
138.5	2.25	2.20	2.06	1.74	1.46	1.24	1.10	1.03	1.01
139.0	2.26	2.20	2.05	1.75	1.46	1.24	1.11	1.03	1.00
139.5	2.25	2.21	2.06	1.74	1.46	1.24	1.10	1.03	1.01
140.0	2.26	2.20	2.05	1.74	1.46	1.23	1.10	1.02	1.00
140.5	2.25	2.21	2.05	1.74	1.46	1.23	1.10	1.02	1.01
141.0	2.26	2.21	2.05	1.74	1.46	1.23	1.10	1.03	1.01
141.5	2.27	2.21	2.07	1.75	1.46	1.24	1.10	1.02	1.01
142.0	2.26	2.21	2.05	1.74	1.46	1.24	1.10	1.03	1.01
142.5	2.27	2.21	2.06	1.74	1.47	1.23	1.09	1.02	1.01
143.0	2.27	2.21	2.06	1.75	1.47	1.23	1.09	1.03	1.00
143.5	2.27	2.21	2.06	1.74	1.46	1.24	1.10	1.03	1.01
144.0	2.28	2.22	2.05	1.74	1.45	1.23	1.10	1.02	1.00
144.5	2.27	2.22	2.06	1.75	1.46	1.23	1.10	1.03	1.00
145.0	2.28	2.22	2.06	1.75	1.47	1.23	1.09	1.02	1.00
145.5	2.26	2.22	2.06	1.73	1.46	1.23	1.09	1.02	1.00
146.0	2.27	2.22	2.06	1.75	1.46	1.23	1.10	1.02	1.01
146.5	2.25	2.22	2.05	1.74	1.46	1.23	1.09	1.02	1.00
147.0	2.28	2.23	2.06	1.75	1.45	1.23	1.10	1.03	1.00
147.5	2.27	2.23	2.07	1.74	1.46	1.22	1.10	1.03	1.01
148.0	2.27	2.22	2.06	1.74	1.46	1.22	1.09	1.03	1.01
148.5	2.27	2.22	2.07	1.75	1.46	1.23	1.10	1.03	1.00
149.0	2.28	2.22	2.07	1.74	1.45	1.22	1.09	1.02	1.01
149.5	2.28	2.23	2.07	1.74	1.46	1.23	1.10	1.03	1.01
150.0	2.27	2.23	2.06	1.74	1.46	1.22	1.09	1.02	1.01
150.5	2.27	2.22	2.06	1.73	1.45	1.22	1.09	1.02	1.00
151.0	2.28	2.23	2.07	1.75	1.46	1.22	1.09	1.01	1.00
151.5	2.27	2.22	2.06	1.75	1.45	1.22	1.09	1.02	1.00
152.0	2.28	2.23	2.07	1.74	1.46	1.22	1.09	1.02	1.01
152.5	2.28	2.23	2.06	1.75	1.46	1.22	1.08	1.02	1.00
153.0	2.29	2.23	2.07	1.75	1.46	1.22	1.09	1.02	1.01
153.5	2.29	2.22	2.07	1.74	1.46	1.22	1.10	1.01	1.00
154.0	2.27	2.23	2.06	1.73	1.45	1.22	1.09	1.02	1.00
154.5	2.29	2.24	2.07	1.74	1.45	1.22	1.09	1.02	1.00
155.0	2.29	2.23	2.06	1.74	1.45	1.21	1.09	1.01	1.00
155.5	2.28	2.23	2.07	1.74	1.45	1.22	1.08	1.02	1.00
156.0	2.29	2.24	2.06	1.74	1.45	1.22	1.09	1.02	1.00
156.5	2.29	2.23	2.06	1.73	1.44	1.21	1.08	1.01	1.00
157.0	2.29	2.23	2.07	1.74	1.46	1.22	1.09	1.01	1.00
157.5	2.29	2.24	2.07	1.75	1.45	1.22	1.09	1.02	1.00
158.0	2.29	2.24	2.08	1.74	1.45	1.21	1.09	1.02	1.00

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
158.5	2.30	2.24	2.07	1.75	1.45	1.22	1.08	1.01	1.01
159.0	2.29	2.23	2.07	1.73	1.45	1.21	1.09	1.01	1.00
159.5	2.29	2.25	2.07	1.74	1.46	1.21	1.09	1.02	1.00
160.0	2.30	2.25	2.07	1.74	1.45	1.21	1.08	1.01	1.00
160.5	2.29	2.24	2.07	1.74	1.45	1.21	1.08	1.01	1.01
161.0	2.31	2.24	2.07	1.74	1.44	1.21	1.08	1.00	1.00
161.5	2.29	2.24	2.07	1.74	1.45	1.21	1.08	1.01	1.00
162.0	2.31	2.25	2.07	1.74	1.45	1.21	1.08	1.00	1.00
162.5	2.30	2.24	2.06	1.74	1.45	1.21	1.08	1.01	1.00
163.0	2.31	2.25	2.07	1.75	1.45	1.21	1.08	1.01	1.01
163.5	2.31	2.25	2.07	1.74	1.45	1.21	1.09	1.01	1.00
164.0	2.30	2.25	2.06	1.74	1.45	1.20	1.08	1.00	1.00
164.5	2.31	2.25	2.08	1.73	1.45	1.21	1.08	1.01	1.00
165.0	2.31	2.25	2.07	1.73	1.45	1.21	1.08	1.01	1.01
165.5	2.30	2.24	2.07	1.74	1.45	1.21	1.08	1.01	1.00
166.0	2.30	2.26	2.08	1.74	1.45	1.20	1.08	1.01	1.00
166.5	2.30	2.25	2.07	1.75	1.44	1.20	1.08	1.00	1.00
167.0	2.31	2.25	2.07	1.74	1.45	1.20	1.07	1.01	1.00
167.5	2.31	2.25	2.07	1.74	1.44	1.21	1.08	1.01	1.00
168.0	2.31	2.25	2.07	1.73	1.44	1.20	1.08	1.00	1.01
168.5	2.30	2.24	2.07	1.74	1.45	1.20	1.08	1.00	0.99
169.0	2.31	2.26	2.07	1.74	1.44	1.20	1.07	1.01	1.00
169.5	2.32	2.24	2.06	1.73	1.44	1.20	1.08	1.00	1.00
170.0	2.31	2.25	2.08	1.74	1.44	1.20	1.08	1.01	1.00
170.5	2.31	2.25	2.07	1.73	1.44	1.20	1.07	1.00	0.99
171.0	2.30	2.26	2.06	1.74	1.44	1.21	1.08	1.01	1.00
171.5	2.31	2.25	2.07	1.74	1.43	1.20	1.07	1.00	1.00
172.0	2.31	2.25	2.07	1.73	1.44	1.19	1.07	1.00	1.00
172.5	2.31	2.25	2.07	1.73	1.44	1.20	1.08	1.00	1.00
173.0	2.31	2.26	2.07	1.73	1.44	1.20	1.07	1.01	1.00
173.5	2.31	2.25	2.07	1.73	1.43	1.20	1.07	1.01	1.00
174.0	2.32	2.26	2.07	1.73	1.44	1.21	1.07	1.00	1.00
174.5	2.31	2.26	2.08	1.73	1.44	1.20	1.08	1.00	1.00
175.0	2.33	2.25	2.07	1.73	1.43	1.19	1.08	1.00	1.00
175.5	2.30	2.25	2.06	1.73	1.43	1.19	1.07	1.00	1.00
176.0	2.32	2.25	2.07	1.73	1.43	1.20	1.07	0.99	1.00
176.5	2.32	2.26	2.07	1.73	1.43	1.19	1.07	1.00	1.00
177.0	2.33	2.26	2.06	1.74	1.43	1.19	1.07	1.00	1.00
177.5	2.32	2.25	2.07	1.73	1.43	1.19	1.07	1.00	1.00
178.0	2.32	2.26	2.08	1.73	1.44	1.19	1.07	1.00	1.00
178.5	2.34	2.26	2.07	1.73	1.43	1.20	1.07	1.00	1.00
179.0	2.32	2.26	2.08	1.74	1.44	1.19	1.07	1.00	1.00
179.5	2.32	2.26	2.07	1.73	1.44	1.20	1.07	1.00	1.00
180.0	2.32	2.26	2.07	1.74	1.43	1.19	1.06	0.99	1.00
180.5	2.32	2.27	2.07	1.73	1.43	1.19	1.07	0.99	1.00
181.0	2.33	2.26	2.08	1.73	1.43	1.19	1.07	0.99	1.00
181.5	2.33	2.25	2.07	1.73	1.44	1.20	1.07	1.00	1.00
182.0	2.32	2.26	2.07	1.73	1.43	1.19	1.06	0.99	1.00
182.5	2.33	2.26	2.08	1.73	1.43	1.19	1.07	1.00	1.00
183.0	2.33	2.27	2.07	1.73	1.43	1.19	1.07	1.00	1.00
183.5	2.34	2.27	2.07	1.72	1.43	1.19	1.06	0.99	1.00
184.0	2.33	2.26	2.07	1.73	1.44	1.19	1.06	0.99	0.99
184.5	2.32	2.27	2.07	1.72	1.43	1.18	1.07	1.00	1.00

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
185.0	2.32	2.26	2.07	1.72	1.43	1.19	1.07	0.99	1.00
185.5	2.34	2.27	2.07	1.72	1.43	1.19	1.07	1.00	1.00
186.0	2.34	2.26	2.07	1.73	1.42	1.19	1.07	0.99	0.99
186.5	2.32	2.27	2.07	1.73	1.43	1.19	1.06	0.99	1.00
187.0	2.33	2.27	2.07	1.73	1.44	1.19	1.07	0.99	0.99
187.5	2.33	2.26	2.06	1.72	1.42	1.18	1.06	0.99	0.99
188.0	2.33	2.27	2.07	1.72	1.43	1.19	1.07	0.99	1.00
188.5	2.33	2.26	2.07	1.73	1.42	1.18	1.07	0.99	1.00
189.0	2.33	2.27	2.07	1.72	1.43	1.18	1.06	0.98	0.99
189.5	2.33	2.27	2.07	1.72	1.42	1.18	1.06	1.00	1.00
190.0	2.33	2.27	2.07	1.72	1.42	1.18	1.06	0.99	1.00
190.5	2.34	2.27	2.07	1.73	1.43	1.19	1.06	0.99	0.99
191.0	2.33	2.27	2.07	1.73	1.42	1.19	1.06	0.99	1.00
191.5	2.32	2.28	2.07	1.72	1.42	1.18	1.05	0.99	0.99
192.0	2.33	2.27	2.06	1.72	1.42	1.18	1.06	0.98	0.99
192.5	2.33	2.27	2.07	1.72	1.42	1.18	1.06	0.98	0.99
193.0	2.34	2.27	2.07	1.73	1.42	1.18	1.06	0.99	1.00
193.5	2.34	2.28	2.06	1.73	1.42	1.18	1.06	0.98	0.99
194.0	2.34	2.27	2.07	1.72	1.43	1.18	1.06	0.99	1.00
194.5	2.33	2.28	2.07	1.72	1.43	1.18	1.06	0.99	1.00
195.0	2.34	2.27	2.08	1.72	1.42	1.19	1.07	0.99	1.00
195.5	2.34	2.27	2.08	1.72	1.42	1.18	1.06	0.98	0.99
196.0	2.35	2.27	2.07	1.71	1.42	1.18	1.06	0.99	0.99
196.5	2.34	2.28	2.06	1.72	1.42	1.18	1.06	0.99	1.00
197.0	2.34	2.27	2.07	1.72	1.42	1.18	1.06	0.98	0.99
197.5	2.33	2.27	2.07	1.72	1.41	1.18	1.05	0.98	0.99
198.0	2.33	2.27	2.05	1.72	1.42	1.18	1.06	0.98	0.99
198.5	2.34	2.27	2.06	1.72	1.41	1.17	1.06	0.98	0.99
199.0	2.34	2.28	2.08	1.72	1.43	1.18	1.05	0.99	1.00
199.5	2.34	2.27	2.07	1.72	1.41	1.17	1.06	0.98	0.99
200.0	2.35	2.28	2.05	1.73	1.42	1.18	1.06	0.98	1.00
200.5	2.34	2.26	2.06	1.71	1.41	1.17	1.05	0.98	0.99
201.0	2.34	2.27	2.07	1.72	1.41	1.18	1.05	0.98	1.00
201.5	2.33	2.28	2.07	1.71	1.42	1.17	1.06	0.99	1.00
202.0	2.35	2.27	2.06	1.72	1.41	1.17	1.05	0.98	0.99
202.5	2.34	2.27	2.07	1.72	1.42	1.18	1.06	0.98	0.99
203.0	2.34	2.27	2.07	1.71	1.41	1.17	1.05	0.98	0.99
203.5	2.35	2.27	2.06	1.72	1.42	1.17	1.05	0.99	0.99
204.0	2.35	2.29	2.08	1.72	1.42	1.18	1.05	0.98	1.00
204.5	2.35	2.27	2.07	1.71	1.41	1.17	1.05	0.98	1.00
205.0	2.33	2.27	2.05	1.72	1.41	1.16	1.04	0.99	0.99
205.5	2.34	2.27	2.06	1.71	1.42	1.17	1.05	0.98	0.99
206.0	2.36	2.27	2.06	1.72	1.40	1.17	1.05	0.97	1.00
206.5	2.33	2.27	2.05	1.71	1.41	1.16	1.05	0.98	0.99
207.0	2.35	2.28	2.06	1.71	1.42	1.17	1.06	0.98	0.99
207.5	2.35	2.27	2.06	1.70	1.41	1.17	1.05	0.97	0.99
208.0	2.34	2.27	2.07	1.70	1.41	1.17	1.04	0.98	0.99
208.5	2.36	2.28	2.06	1.72	1.41	1.17	1.05	0.98	0.99
209.0	2.35	2.28	2.06	1.71	1.41	1.17	1.05	0.98	0.99
209.5	2.35	2.28	2.07	1.72	1.41	1.16	1.04	0.98	1.00
210.0	2.35	2.27	2.06	1.71	1.40	1.16	1.05	0.97	0.99
210.5	2.35	2.28	2.06	1.71	1.41	1.17	1.05	0.97	0.99
211.0	2.35	2.28	2.07	1.71	1.41	1.16	1.05	0.97	1.00

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
211.5	2.35	2.27	2.06	1.70	1.40	1.17	1.05	0.97	0.99
212.0	2.35	2.28	2.07	1.72	1.41	1.18	1.04	0.98	1.00
212.5	2.35	2.29	2.07	1.71	1.41	1.16	1.05	0.97	0.99
213.0	2.35	2.28	2.06	1.71	1.40	1.16	1.05	0.98	0.99
213.5	2.36	2.27	2.06	1.71	1.41	1.16	1.05	0.98	1.00
214.0	2.34	2.27	2.06	1.71	1.41	1.16	1.05	0.98	0.99
214.5	2.36	2.28	2.06	1.71	1.40	1.17	1.05	0.98	1.00
215.0	2.35	2.27	2.06	1.71	1.41	1.17	1.05	0.97	0.99
215.5	2.35	2.28	2.06	1.70	1.40	1.16	1.04	0.97	1.00
216.0	2.35	2.28	2.05	1.70	1.40	1.16	1.05	0.97	0.99
216.5	2.34	2.28	2.07	1.70	1.40	1.16	1.05	0.97	0.99
217.0	2.35	2.28	2.06	1.70	1.39	1.16	1.04	0.97	0.99
217.5	2.35	2.28	2.05	1.69	1.39	1.16	1.03	0.97	0.99
218.0	2.36	2.27	2.06	1.71	1.41	1.16	1.04	0.98	0.99
218.5	2.36	2.28	2.07	1.71	1.40	1.17	1.05	0.97	0.99
219.0	2.34	2.28	2.05	1.69	1.40	1.15	1.04	0.97	0.99
219.5	2.35	2.28	2.06	1.70	1.40	1.16	1.04	0.98	1.00
220.0	2.35	2.27	2.06	1.69	1.40	1.16	1.03	0.97	0.99
220.5	2.35	2.28	2.05	1.69	1.40	1.16	1.04	0.97	0.99
221.0	2.35	2.27	2.05	1.70	1.40	1.15	1.04	0.97	0.99
221.5	2.34	2.27	2.06	1.71	1.39	1.16	1.04	0.97	0.99
222.0	2.36	2.28	2.05	1.70	1.39	1.15	1.04	0.97	1.00
222.5	2.36	2.27	2.05	1.69	1.40	1.16	1.04	0.97	0.99
223.0	2.36	2.28	2.05	1.70	1.40	1.16	1.03	0.97	0.99
223.5	2.35	2.27	2.05	1.70	1.40	1.15	1.04	0.97	0.99
224.0	2.35	2.29	2.05	1.70	1.39	1.16	1.04	0.97	0.99
224.5	2.36	2.28	2.05	1.69	1.39	1.16	1.04	0.97	1.00
225.0	2.36	2.29	2.06	1.70	1.39	1.16	1.04	0.97	1.00
225.5	2.36	2.29	2.04	1.70	1.41	1.16	1.03	0.97	0.99
226.0	2.35	2.28	2.06	1.71	1.40	1.16	1.04	0.97	0.99
226.5	2.36	2.28	2.05	1.70	1.39	1.16	1.04	0.97	1.00
227.0	2.36	2.29	2.05	1.69	1.40	1.16	1.04	0.97	1.00
227.5	2.35	2.27	2.05	1.69	1.39	1.16	1.03	0.97	0.99
228.0	2.36	2.29	2.04	1.70	1.39	1.15	1.03	0.96	0.99
228.5	2.35	2.27	2.04	1.69	1.39	1.15	1.03	0.97	0.99
229.0	2.36	2.29	2.05	1.70	1.39	1.15	1.03	0.97	0.99
229.5	2.35	2.28	2.04	1.69	1.39	1.14	1.03	0.96	0.99
230.0	2.36	2.28	2.05	1.70	1.40	1.15	1.04	0.97	0.99
230.5	2.35	2.28	2.04	1.70	1.39	1.15	1.03	0.97	1.00
231.0	2.35	2.28	2.05	1.69	1.38	1.15	1.03	0.96	0.99
231.5	2.35	2.27	2.04	1.69	1.39	1.15	1.03	0.96	0.99
232.0	2.35	2.29	2.04	1.69	1.39	1.16	1.03	0.97	0.99
232.5	2.36	2.28	2.05	1.69	1.39	1.15	1.03	0.97	0.99
233.0	2.36	2.28	2.05	1.69	1.39	1.15	1.03	0.97	0.99
233.5	2.36	2.28	2.04	1.69	1.39	1.16	1.03	0.96	0.99
234.0	2.36	2.28	2.04	1.69	1.38	1.15	1.03	0.97	0.99
234.5	2.36	2.28	2.03	1.69	1.38	1.14	1.03	0.97	0.99
235.0	2.35	2.27	2.03	1.68	1.38	1.15	1.03	0.97	0.99
235.5	2.36	2.27	2.04	1.68	1.39	1.15	1.03	0.96	0.99
236.0	2.36	2.28	2.05	1.69	1.39	1.15	1.03	0.97	0.99
236.5	2.36	2.27	2.04	1.69	1.39	1.15	1.02	0.96	0.99
237.0	2.35	2.28	2.05	1.69	1.39	1.15	1.03	0.96	0.99
237.5	2.35	2.29	2.05	1.70	1.38	1.15	1.02	0.97	0.99

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
238.0	2.35	2.28	2.04	1.68	1.38	1.15	1.03	0.95	0.99
238.5	2.35	2.28	2.04	1.68	1.39	1.15	1.03	0.96	0.99
239.0	2.36	2.28	2.05	1.69	1.38	1.15	1.03	0.97	1.00
239.5	2.36	2.27	2.03	1.68	1.39	1.15	1.03	0.97	0.99
240.0	2.36	2.29	2.04	1.68	1.39	1.15	1.03	0.96	0.99
240.5	2.36	2.28	2.04	1.68	1.39	1.14	1.03	0.96	0.99
241.0	2.37	2.27	2.03	1.68	1.38	1.15	1.02	0.96	0.99
241.5	2.35	2.27	2.04	1.69	1.37	1.15	1.04	0.96	0.99
242.0	2.35	2.28	2.03	1.68	1.38	1.15	1.03	0.96	0.99
242.5	2.37	2.28	2.04	1.69	1.38	1.15	1.03	0.96	0.99
243.0	2.36	2.28	2.03	1.70	1.39	1.14	1.03	0.96	0.99
243.5	2.36	2.28	2.04	1.68	1.38	1.14	1.03	0.96	0.99
244.0	2.36	2.28	2.04	1.69	1.38	1.15	1.03	0.96	0.99
244.5	2.36	2.28	2.04	1.69	1.38	1.15	1.02	0.96	0.99
245.0	2.36	2.28	2.04	1.69	1.38	1.15	1.03	0.96	0.99
245.5	2.36	2.29	2.03	1.68	1.39	1.14	1.03	0.97	0.98
246.0	2.37	2.27	2.03	1.68	1.38	1.15	1.02	0.96	0.99
246.5	2.37	2.29	2.03	1.68	1.38	1.14	1.02	0.96	0.99
247.0	2.36	2.27	2.03	1.68	1.38	1.14	1.02	0.96	0.99
247.5	2.37	2.28	2.04	1.69	1.38	1.14	1.02	0.96	0.99
248.0	2.37	2.27	2.03	1.68	1.38	1.14	1.02	0.96	0.99
248.5	2.36	2.28	2.02	1.68	1.37	1.15	1.02	0.96	0.99
249.0	2.37	2.28	2.03	1.67	1.38	1.15	1.02	0.96	0.99
249.5	2.36	2.26	2.02	1.68	1.37	1.14	1.03	0.96	0.98
250.0	2.35	2.27	2.04	1.67	1.37	1.14	1.02	0.95	0.99
250.5	2.36	2.27	2.04	1.68	1.39	1.14	1.02	0.96	0.99
251.0	2.37	2.28	2.03	1.68	1.38	1.14	1.02	0.96	0.99
251.5	2.36	2.27	2.03	1.68	1.37	1.14	1.02	0.95	0.99
252.0	2.36	2.28	2.03	1.67	1.37	1.14	1.02	0.95	0.99
252.5	2.36	2.28	2.03	1.67	1.39	1.14	1.03	0.96	0.99
253.0	2.37	2.28	2.03	1.68	1.37	1.14	1.02	0.95	0.99
253.5	2.37	2.28	2.03	1.67	1.38	1.14	1.02	0.95	0.99
254.0	2.36	2.27	2.03	1.68	1.38	1.14	1.02	0.96	0.99
254.5	2.36	2.27	2.02	1.67	1.38	1.14	1.02	0.95	0.98
255.0	2.37	2.29	2.03	1.68	1.38	1.14	1.02	0.96	0.99
255.5	2.36	2.28	2.02	1.68	1.37	1.13	1.02	0.95	0.99
256.0	2.36	2.28	2.04	1.68	1.38	1.14	1.02	0.97	0.99
256.5	2.36	2.28	2.02	1.68	1.37	1.14	1.02	0.95	0.99
257.0	2.36	2.27	2.02	1.67	1.37	1.13	1.02	0.95	0.98
257.5	2.36	2.28	2.03	1.68	1.37	1.13	1.02	0.96	0.99
258.0	2.37	2.27	2.02	1.67	1.37	1.13	1.02	0.95	0.98
258.5	2.36	2.27	2.03	1.67	1.37	1.13	1.01	0.95	0.99
259.0	2.37	2.27	2.03	1.67	1.38	1.14	1.02	0.95	0.99
259.5	2.37	2.27	2.02	1.67	1.36	1.13	1.02	0.95	0.99
260.0	2.36	2.27	2.02	1.67	1.36	1.14	1.02	0.95	0.99
260.5	2.36	2.28	2.02	1.67	1.37	1.14	1.01	0.96	0.99
261.0	2.37	2.28	2.02	1.67	1.36	1.14	1.01	0.96	0.99
261.5	2.36	2.27	2.02	1.68	1.37	1.13	1.02	0.95	0.99
262.0	2.37	2.26	2.02	1.67	1.37	1.13	1.01	0.96	0.99
262.5	2.37	2.28	2.01	1.67	1.37	1.13	1.02	0.96	0.99
263.0	2.36	2.27	2.02	1.67	1.36	1.14	1.01	0.95	0.99
263.5	2.36	2.28	2.02	1.68	1.37	1.14	1.02	0.95	0.99
264.0	2.36	2.28	2.01	1.67	1.37	1.13	1.02	0.95	0.99

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
264.5	2.37	2.30	2.03	1.67	1.38	1.14	1.01	0.95	1.00
265.0	2.37	2.27	2.02	1.67	1.36	1.13	1.02	0.95	0.99
265.5	2.36	2.27	2.02	1.67	1.37	1.13	1.01	0.95	0.99
266.0	2.36	2.27	2.01	1.67	1.36	1.13	1.01	0.95	0.99
266.5	2.37	2.27	2.01	1.66	1.37	1.13	1.01	0.95	0.98
267.0	2.36	2.28	2.01	1.66	1.36	1.13	1.00	0.95	0.99
267.5	2.36	2.27	2.01	1.67	1.36	1.13	1.02	0.95	0.98
268.0	2.36	2.28	2.02	1.67	1.37	1.13	1.01	0.95	0.99
268.5	2.37	2.28	2.02	1.67	1.37	1.14	1.02	0.96	0.99
269.0	2.37	2.28	2.02	1.67	1.36	1.14	1.01	0.95	0.99
269.5	2.35	2.27	2.02	1.66	1.36	1.14	1.02	0.95	0.99
270.0	2.36	2.27	2.01	1.66	1.36	1.13	1.02	0.95	0.99
270.5	2.37	2.27	2.02	1.66	1.37	1.12	1.02	0.95	0.99
271.0	2.36	2.27	2.02	1.67	1.37	1.13	1.01	0.95	0.98
271.5	2.36	2.27	2.01	1.66	1.36	1.13	1.01	0.95	0.99
272.0	2.36	2.28	2.01	1.66	1.37	1.13	1.02	0.95	0.99
272.5	2.36	2.27	2.02	1.66	1.37	1.13	1.02	0.95	0.99
273.0	2.37	2.28	2.02	1.66	1.36	1.13	1.00	0.94	0.99
273.5	2.36	2.27	2.01	1.66	1.36	1.13	1.01	0.95	0.98
274.0	2.36	2.27	2.02	1.66	1.36	1.13	1.01	0.95	0.99
274.5	2.37	2.28	2.02	1.66	1.36	1.13	1.01	0.95	0.99
275.0	2.37	2.27	2.01	1.66	1.36	1.13	1.01	0.95	0.99
275.5	2.35	2.27	2.00	1.66	1.36	1.13	1.01	0.94	0.98
276.0	2.36	2.27	2.01	1.66	1.36	1.12	1.00	0.95	0.99
276.5	2.36	2.27	2.01	1.65	1.36	1.13	1.01	0.95	0.98
277.0	2.36	2.28	2.02	1.67	1.36	1.13	1.01	0.95	0.99
277.5	2.36	2.27	2.01	1.67	1.35	1.13	1.00	0.95	0.99
278.0	2.36	2.27	2.01	1.66	1.36	1.14	1.01	0.94	0.99
278.5	2.35	2.26	2.00	1.66	1.35	1.12	1.00	0.94	0.99
279.0	2.36	2.27	2.01	1.66	1.35	1.13	1.01	0.94	0.98
279.5	2.36	2.27	2.01	1.65	1.36	1.12	1.01	0.95	0.98
280.0	2.36	2.27	1.99	1.65	1.35	1.12	1.01	0.94	0.99
280.5	2.37	2.27	2.00	1.66	1.35	1.12	1.00	0.94	0.99
281.0	2.36	2.27	2.00	1.64	1.35	1.12	1.00	0.95	0.98
281.5	2.37	2.28	2.00	1.64	1.36	1.12	1.00	0.95	0.98
282.0	2.37	2.26	2.00	1.65	1.35	1.13	1.01	0.94	0.99
282.5	2.37	2.27	2.00	1.65	1.35	1.12	1.00	0.94	0.98
283.0	2.35	2.27	2.00	1.65	1.35	1.12	1.00	0.94	0.98
283.5	2.35	2.27	2.00	1.65	1.35	1.13	1.01	0.94	0.99
284.0	2.35	2.26	2.00	1.65	1.35	1.12	1.01	0.94	0.99
284.5	2.36	2.28	2.00	1.65	1.35	1.13	1.00	0.94	0.99
285.0	2.36	2.26	2.00	1.65	1.35	1.12	1.00	0.94	0.99
285.5	2.36	2.27	1.99	1.66	1.34	1.12	1.00	0.94	0.99
286.0	2.37	2.28	2.00	1.65	1.36	1.13	1.00	0.94	0.98
286.5	2.36	2.27	1.99	1.65	1.35	1.12	1.00	0.94	0.98
287.0	2.37	2.26	1.99	1.66	1.35	1.12	1.00	0.94	0.98
287.5	2.37	2.27	2.00	1.64	1.34	1.12	1.01	0.94	0.98
288.0	2.36	2.27	2.00	1.65	1.35	1.12	1.00	0.94	0.98
288.5	2.38	2.27	2.00	1.64	1.36	1.13	1.01	0.94	0.99
289.0	2.37	2.27	2.00	1.65	1.36	1.13	1.00	0.94	0.98
289.5	2.35	2.26	1.99	1.64	1.34	1.11	1.00	0.93	0.99
290.0	2.36	2.27	2.00	1.65	1.34	1.12	1.00	0.94	0.98
290.5	2.35	2.27	1.99	1.64	1.34	1.12	1.00	0.94	0.98

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
291.0	2.36	2.26	2.00	1.64	1.35	1.12	1.00	0.94	0.98
291.5	2.35	2.27	1.99	1.64	1.34	1.12	1.00	0.94	0.99
292.0	2.37	2.26	2.00	1.64	1.34	1.12	1.00	0.94	0.98
292.5	2.37	2.27	2.00	1.65	1.34	1.11	1.00	0.94	0.98
293.0	2.36	2.26	1.99	1.65	1.35	1.12	1.00	0.94	0.98
293.5	2.37	2.26	2.00	1.64	1.36	1.12	1.00	0.94	0.98
294.0	2.36	2.27	2.00	1.64	1.35	1.12	1.00	0.93	0.99
294.5	2.36	2.27	1.99	1.64	1.34	1.11	1.00	0.93	0.98
295.0	2.36	2.27	2.00	1.65	1.35	1.12	1.00	0.93	0.99
295.5	2.36	2.27	1.99	1.65	1.34	1.12	1.00	0.94	0.99
296.0	2.36	2.26	1.99	1.64	1.35	1.13	1.00	0.94	0.98
296.5	2.36	2.25	1.99	1.64	1.34	1.11	1.00	0.93	0.98
297.0	2.36	2.27	2.00	1.64	1.35	1.12	0.99	0.94	0.98
297.5	2.36	2.27	1.99	1.64	1.34	1.11	1.00	0.94	0.99
298.0	2.35	2.26	1.99	1.64	1.33	1.12	1.00	0.94	0.98
298.5	2.36	2.26	2.00	1.64	1.35	1.11	0.99	0.94	0.99
299.0	2.36	2.26	1.99	1.64	1.34	1.12	1.00	0.94	0.99
299.5	2.36	2.26	1.99	1.65	1.34	1.12	0.99	0.94	0.99
300.0	2.35	2.26	1.98	1.62	1.34	1.11	0.99	0.93	0.98
300.5	2.35	2.26	1.99	1.64	1.34	1.12	1.00	0.94	0.98
301.0	2.36	2.26	1.98	1.64	1.35	1.11	1.00	0.93	0.98
301.5	2.35	2.26	1.99	1.64	1.33	1.12	0.99	0.93	0.98
302.0	2.36	2.25	1.98	1.63	1.34	1.11	0.99	0.93	0.98
302.5	2.36	2.26	2.00	1.64	1.34	1.12	1.00	0.94	0.99
303.0	2.36	2.26	1.99	1.63	1.34	1.11	0.99	0.93	0.99
303.5	2.35	2.26	1.98	1.63	1.34	1.12	0.99	0.94	0.99
304.0	2.36	2.26	1.98	1.64	1.34	1.12	0.99	0.93	0.98
304.5	2.36	2.26	1.99	1.65	1.35	1.12	0.99	0.94	0.98
305.0	2.37	2.27	1.99	1.64	1.34	1.12	1.00	0.93	0.98
305.5	2.36	2.25	1.99	1.63	1.34	1.12	1.00	0.94	0.98
306.0	2.37	2.26	1.98	1.63	1.34	1.11	1.00	0.93	0.98
306.5	2.35	2.27	1.99	1.63	1.34	1.11	0.99	0.93	0.99
307.0	2.36	2.26	1.99	1.64	1.34	1.11	1.00	0.94	0.98
307.5	2.35	2.26	1.98	1.64	1.33	1.11	0.99	0.93	0.98
308.0	2.36	2.26	1.98	1.64	1.34	1.12	1.00	0.94	0.98
308.5	2.36	2.25	1.98	1.63	1.33	1.11	0.99	0.94	0.98
309.0	2.36	2.25	1.98	1.63	1.34	1.11	0.99	0.93	0.98
309.5	2.35	2.25	1.98	1.63	1.34	1.11	0.99	0.93	0.98
310.0	2.36	2.25	1.98	1.63	1.34	1.11	0.99	0.93	0.99
310.5	2.37	2.26	1.97	1.63	1.33	1.11	0.99	0.93	0.98
311.0	2.37	2.26	1.98	1.63	1.34	1.10	1.00	0.93	0.98
311.5	2.34	2.25	1.98	1.63	1.33	1.11	0.99	0.93	0.98
312.0	2.34	2.26	1.97	1.64	1.33	1.11	0.98	0.93	0.99
312.5	2.36	2.26	1.98	1.64	1.34	1.11	0.98	0.93	0.99
313.0	2.35	2.26	1.98	1.63	1.33	1.11	0.99	0.93	0.99
313.5	2.36	2.26	1.98	1.62	1.33	1.11	0.99	0.93	0.98
314.0	2.35	2.26	1.98	1.63	1.33	1.11	0.99	0.93	0.99
314.5	2.37	2.25	1.98	1.63	1.34	1.11	0.99	0.93	0.98
315.0	2.36	2.26	1.98	1.63	1.33	1.11	0.98	0.93	0.98
315.5	2.36	2.26	1.98	1.64	1.34	1.11	0.99	0.93	0.98
316.0	2.36	2.25	1.97	1.62	1.33	1.11	1.00	0.93	0.98
316.5	2.36	2.26	1.98	1.63	1.33	1.11	0.99	0.93	0.98
317.0	2.35	2.25	1.98	1.63	1.33	1.11	0.99	0.93	0.98

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
317.5	2.36	2.25	1.98	1.64	1.34	1.11	0.99	0.94	0.98
318.0	2.37	2.26	1.98	1.63	1.33	1.11	0.99	0.93	0.98
318.5	2.36	2.26	1.98	1.63	1.33	1.11	0.99	0.93	0.98
319.0	2.35	2.26	1.98	1.62	1.33	1.11	0.99	0.92	0.98
319.5	2.37	2.25	1.97	1.63	1.33	1.11	0.99	0.93	0.98
320.0	2.36	2.26	1.97	1.63	1.33	1.11	0.99	0.93	0.98
320.5	2.35	2.25	1.97	1.62	1.32	1.11	0.99	0.93	0.99
321.0	2.35	2.26	1.97	1.62	1.33	1.11	0.99	0.93	0.98
321.5	2.36	2.25	1.97	1.62	1.33	1.11	0.99	0.92	0.98
322.0	2.36	2.26	1.98	1.63	1.33	1.12	0.99	0.93	0.99
322.5	2.35	2.25	1.97	1.62	1.33	1.11	0.99	0.92	0.99
323.0	2.36	2.26	1.97	1.62	1.33	1.10	0.99	0.92	0.98
323.5	2.37	2.26	1.97	1.62	1.33	1.11	0.98	0.93	0.98
324.0	2.35	2.25	1.98	1.63	1.33	1.11	0.98	0.93	0.98
324.5	2.36	2.25	1.97	1.62	1.32	1.10	0.99	0.93	0.98
325.0	2.36	2.25	1.97	1.62	1.33	1.11	0.99	0.92	0.98
325.5	2.36	2.25	1.96	1.61	1.32	1.10	0.98	0.92	0.98
326.0	2.35	2.25	1.97	1.63	1.33	1.10	0.98	0.92	0.98
326.5	2.36	2.25	1.97	1.61	1.33	1.10	0.99	0.92	0.98
327.0	2.36	2.25	1.96	1.62	1.33	1.11	0.99	0.93	0.99
327.5	2.34	2.25	1.97	1.61	1.33	1.10	0.99	0.92	0.98
328.0	2.35	2.24	1.96	1.62	1.32	1.11	0.99	0.92	0.98
328.5	2.36	2.26	1.96	1.62	1.33	1.11	0.98	0.92	0.98
329.0	2.35	2.25	1.95	1.61	1.32	1.10	0.98	0.92	0.98
329.5	2.36	2.26	1.97	1.62	1.32	1.11	0.98	0.93	0.98
330.0	2.35	2.25	1.96	1.62	1.32	1.11	0.98	0.92	0.98
330.5	2.35	2.25	1.96	1.62	1.33	1.11	0.98	0.92	0.98
331.0	2.35	2.25	1.96	1.62	1.32	1.11	0.98	0.92	0.98
331.5	2.35	2.26	1.96	1.61	1.32	1.10	0.98	0.92	0.98
332.0	2.34	2.26	1.96	1.62	1.33	1.11	0.98	0.92	0.98
332.5	2.35	2.24	1.96	1.61	1.32	1.10	0.98	0.92	0.98
333.0	2.35	2.25	1.96	1.61	1.32	1.11	0.99	0.93	0.98
333.5	2.35	2.24	1.96	1.62	1.33	1.11	0.99	0.92	0.98
334.0	2.35	2.24	1.95	1.62	1.33	1.10	0.99	0.92	0.98
334.5	2.36	2.26	1.97	1.62	1.33	1.10	0.99	0.92	0.98
335.0	2.35	2.25	1.96	1.61	1.32	1.10	0.97	0.92	0.97
335.5	2.36	2.25	1.96	1.61	1.33	1.10	0.98	0.92	0.98
336.0	2.35	2.25	1.95	1.62	1.32	1.10	0.98	0.92	0.98
336.5	2.35	2.24	1.95	1.62	1.32	1.11	0.98	0.93	0.98
337.0	2.35	2.25	1.96	1.61	1.32	1.11	0.98	0.92	0.98
337.5	2.35	2.24	1.95	1.61	1.31	1.11	0.98	0.92	0.98
338.0	2.36	2.25	1.96	1.61	1.32	1.10	0.98	0.92	0.98
338.5	2.35	2.24	1.95	1.62	1.33	1.10	0.99	0.93	0.98
339.0	2.35	2.25	1.96	1.62	1.31	1.10	0.98	0.93	0.98
339.5	2.36	2.24	1.96	1.62	1.32	1.11	0.99	0.92	0.99
340.0	2.37	2.24	1.96	1.62	1.32	1.11	0.98	0.93	0.98
340.5	2.35	2.25	1.95	1.62	1.32	1.10	0.98	0.92	0.98
341.0	2.34	2.26	1.95	1.61	1.32	1.11	0.98	0.92	0.98
341.5	2.36	2.24	1.96	1.61	1.31	1.10	0.98	0.92	0.98
342.0	2.35	2.24	1.96	1.62	1.32	1.11	0.98	0.92	0.98
342.5	2.34	2.25	1.96	1.61	1.32	1.11	0.98	0.92	0.98
343.0	2.36	2.25	1.96	1.62	1.32	1.11	0.98	0.92	0.98
343.5	2.35	2.24	1.96	1.60	1.31	1.10	0.98	0.92	0.98

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
344.0	2.35	2.25	1.96	1.61	1.32	1.11	0.98	0.92	0.98
344.5	2.35	2.24	1.95	1.60	1.31	1.10	0.97	0.92	0.98
345.0	2.35	2.25	1.94	1.61	1.32	1.10	0.98	0.92	0.98
345.5	2.34	2.24	1.95	1.60	1.32	1.10	0.98	0.92	0.98
346.0	2.35	2.25	1.95	1.61	1.32	1.10	0.99	0.92	0.98
346.5	2.36	2.24	1.94	1.60	1.31	1.10	0.98	0.92	0.98
347.0	2.35	2.24	1.94	1.60	1.32	1.10	0.98	0.92	0.98
347.5	2.34	2.23	1.95	1.62	1.31	1.10	0.97	0.91	0.98
348.0	2.35	2.25	1.96	1.61	1.32	1.11	0.98	0.93	0.99
348.5	2.35	2.24	1.95	1.61	1.32	1.10	0.98	0.92	0.98
349.0	2.34	2.24	1.96	1.60	1.31	1.10	0.98	0.92	0.98
349.5	2.35	2.25	1.95	1.61	1.32	1.10	0.98	0.91	0.98
350.0	2.35	2.25	1.96	1.60	1.31	1.10	0.98	0.92	0.98
350.5	2.35	2.25	1.95	1.61	1.32	1.11	0.98	0.92	0.98
351.0	2.35	2.23	1.94	1.61	1.32	1.10	0.98	0.92	0.98
351.5	2.34	2.25	1.95	1.61	1.31	1.10	0.98	0.92	0.98
352.0	2.35	2.24	1.95	1.61	1.31	1.09	0.98	0.92	0.98
352.5	2.34	2.23	1.94	1.61	1.32	1.10	0.97	0.92	0.98
353.0	2.35	2.25	1.95	1.61	1.31	1.11	0.98	0.92	0.99
353.5	2.35	2.24	1.94	1.61	1.31	1.10	0.98	0.92	0.98
354.0	2.35	2.24	1.95	1.60	1.32	1.10	0.97	0.92	0.98
354.5	2.35	2.24	1.95	1.61	1.31	1.10	0.98	0.92	0.98
355.0	2.34	2.23	1.94	1.60	1.32	1.10	0.98	0.92	0.99
355.5	2.35	2.23	1.94	1.61	1.31	1.10	0.98	0.92	0.98
356.0	2.34	2.24	1.94	1.60	1.31	1.10	0.97	0.92	0.98
356.5	2.35	2.24	1.95	1.61	1.32	1.11	0.97	0.92	0.98
357.0	2.35	2.24	1.94	1.59	1.32	1.09	0.97	0.91	0.98
357.5	2.35	2.23	1.95	1.60	1.31	1.09	0.97	0.92	0.98
358.0	2.36	2.24	1.94	1.60	1.31	1.10	0.97	0.92	0.98
358.5	2.35	2.25	1.95	1.60	1.31	1.10	0.97	0.92	0.97
359.0	2.34	2.24	1.94	1.61	1.31	1.10	0.97	0.92	0.97
359.5	2.35	2.25	1.95	1.60	1.31	1.10	0.98	0.92	0.98
360.0	2.34	2.24	1.95	1.60	1.30	1.10	0.97	0.92	0.98
360.5	2.35	2.24	1.94	1.60	1.31	1.09	0.98	0.92	0.98
361.0	2.35	2.23	1.94	1.60	1.31	1.10	0.98	0.91	0.98
361.5	2.34	2.24	1.94	1.60	1.31	1.09	0.97	0.92	0.98
362.0	2.35	2.24	1.94	1.59	1.30	1.10	0.97	0.92	0.98
362.5	2.34	2.24	1.95	1.60	1.30	1.10	0.97	0.92	0.97
363.0	2.33	2.24	1.94	1.60	1.30	1.09	0.97	0.91	0.98
363.5	2.34	2.23	1.94	1.59	1.30	1.09	0.97	0.91	0.97
364.0	2.35	2.24	1.93	1.60	1.31	1.10	0.97	0.91	0.98
364.5	2.33	2.23	1.94	1.60	1.30	1.09	0.97	0.92	0.98
365.0	2.35	2.24	1.94	1.60	1.30	1.10	0.97	0.91	0.98
365.5	2.34	2.24	1.94	1.60	1.31	1.10	0.98	0.91	0.98
366.0	2.34	2.23	1.94	1.60	1.31	1.10	0.97	0.91	0.98
366.5	2.34	2.23	1.94	1.61	1.31	1.09	0.98	0.92	0.98
367.0	2.34	2.22	1.93	1.59	1.31	1.10	0.98	0.91	0.98
367.5	2.35	2.24	1.94	1.60	1.31	1.10	0.97	0.91	0.98
368.0	2.33	2.23	1.94	1.59	1.31	1.09	0.97	0.92	0.97
368.5	2.35	2.23	1.94	1.60	1.31	1.10	0.98	0.92	0.98
369.0	2.34	2.23	1.93	1.59	1.31	1.09	0.97	0.91	0.98
369.5	2.34	2.24	1.93	1.59	1.31	1.10	0.98	0.91	0.98
370.0	2.34	2.23	1.94	1.60	1.30	1.09	0.97	0.92	0.98

TABLE 7
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
370.5	2.34	2.23	1.94	1.59	1.30	1.09	0.97	0.92	0.98
371.0	2.33	2.24	1.93	1.59	1.30	1.09	0.97	0.91	0.98
371.5	2.34	2.23	1.93	1.59	1.31	1.09	0.97	0.91	0.97
372.0	2.34	2.24	1.93	1.59	1.31	1.09	0.97	0.91	0.98
372.5	2.34	2.23	1.92	1.60	1.31	1.09	0.97	0.91	0.98
373.0	2.34	2.23	1.94	1.59	1.30	1.09	0.97	0.91	0.98
373.5	2.35	2.23	1.93	1.59	1.30	1.09	0.97	0.91	0.98
374.0	2.33	2.22	1.93	1.59	1.30	1.09	0.97	0.91	0.98
374.5	2.35	2.23	1.93	1.60	1.30	1.10	0.97	0.91	0.98
375.0	2.34	2.23	1.94	1.58	1.30	1.09	0.97	0.91	0.98
375.5	2.33	2.23	1.93	1.59	1.30	1.09	0.97	0.91	0.98
376.0	2.34	2.24	1.93	1.59	1.31	1.09	0.97	0.91	0.98
376.5	2.34	2.23	1.94	1.59	1.30	1.09	0.97	0.91	0.98
377.0	2.34	2.23	1.93	1.59	1.30	1.10	0.97	0.91	0.97
377.5	2.34	2.23	1.92	1.59	1.30	1.09	0.97	0.91	0.98
378.0	2.33	2.22	1.92	1.59	1.30	1.09	0.96	0.91	0.97
378.5	2.34	2.24	1.93	1.59	1.30	1.09	0.97	0.92	0.98
379.0	2.34	2.23	1.92	1.58	1.30	1.09	0.98	0.91	0.97
379.5	2.34	2.22	1.93	1.59	1.31	1.10	0.97	0.91	0.98
380.0	2.34	2.23	1.92	1.58	1.30	1.10	0.97	0.92	0.98
380.5	2.33	2.22	1.93	1.58	1.30	1.09	0.97	0.91	0.97
381.0	2.35	2.23	1.93	1.59	1.31	1.10	0.97	0.91	0.97
381.5	2.33	2.22	1.93	1.59	1.29	1.10	0.97	0.90	0.97
382.0	2.34	2.22	1.92	1.58	1.30	1.09	0.97	0.91	0.98
382.5	2.35	2.23	1.94	1.60	1.30	1.09	0.97	0.91	0.98
383.0	2.35	2.22	1.93	1.58	1.30	1.09	0.97	0.91	0.98
383.5	2.34	2.23	1.92	1.59	1.30	1.09	0.97	0.91	0.98
384.0	2.33	2.22	1.93	1.58	1.29	1.09	0.96	0.91	0.98
384.5	2.34	2.23	1.92	1.58	1.30	1.09	0.97	0.91	0.98
385.0	2.34	2.22	1.92	1.59	1.30	1.09	0.96	0.91	0.98
385.5	2.34	2.23	1.93	1.59	1.30	1.10	0.97	0.91	0.98
386.0	2.33	2.22	1.92	1.58	1.29	1.09	0.97	0.91	0.98
386.5	2.34	2.23	1.92	1.58	1.30	1.09	0.97	0.91	0.98
387.0	2.33	2.23	1.92	1.58	1.30	1.09	0.96	0.91	0.98
387.5	2.34	2.23	1.93	1.59	1.30	1.08	0.97	0.91	0.98
388.0	2.34	2.22	1.92	1.59	1.30	1.09	0.97	0.90	0.98
388.5	2.33	2.23	1.93	1.57	1.30	1.10	0.96	0.91	0.98
389.0	2.33	2.22	1.92	1.58	1.29	1.09	0.96	0.90	0.97
389.5	2.33	2.22	1.93	1.58	1.30	1.09	0.96	0.91	0.97
390.0	2.33	2.22	1.91	1.58	1.30	1.09	0.96	0.90	0.98
390.5	2.34	2.23	1.93	1.59	1.29	1.09	0.97	0.91	0.98
391.0	2.33	2.22	1.92	1.58	1.29	1.10	0.96	0.91	0.98
391.5	2.34	2.22	1.91	1.58	1.28	1.09	0.97	0.91	0.98
392.0	2.33	2.22	1.92	1.58	1.29	1.09	0.96	0.90	0.97
392.5	2.33	2.22	1.93	1.59	1.29	1.09	0.96	0.91	0.98
393.0	2.33	2.23	1.91	1.58	1.29	1.08	0.96	0.90	0.98
393.5	2.34	2.23	1.92	1.58	1.29	1.09	0.97	0.91	0.97
394.0	2.33	2.22	1.91	1.58	1.30	1.10	0.96	0.91	0.98
394.5	2.34	2.23	1.92	1.58	1.29	1.09	0.97	0.91	0.97
395.0	2.33	2.22	1.92	1.57	1.29	1.09	0.96	0.90	0.98
395.5	2.33	2.22	1.91	1.57	1.30	1.10	0.96	0.91	0.97
396.0	2.34	2.23	1.91	1.57	1.30	1.09	0.97	0.91	0.98
396.5	2.34	2.22	1.92	1.59	1.29	1.08	0.97	0.91	0.98

TABLE 7									
Ligand concentration dependency of NMDAR-mediated calcium flux (NR1/2A)									
time sec	100.00 μ M	50.00 μ M	25.00 μ M	12.50 μ M	6.25 μ M	3.13 μ M	1.67 μ M	0.84 μ M	no ligand
397.0	2.33	2.22	1.91	1.58	1.30	1.09	0.96	0.90	0.98
397.5	2.34	2.21	1.91	1.57	1.30	1.09	0.96	0.90	0.98
398.0	2.35	2.22	1.91	1.57	1.29	1.08	0.96	0.91	0.98
398.5	2.34	2.22	1.92	1.58	1.29	1.09	0.97	0.90	0.97
399.0	2.34	2.22	1.90	1.58	1.29	1.08	0.97	0.90	0.97
Data and concentrations rounded to two decimal places									

- [096]** MK801 or DMSO was added and NMDAR-mediated calcium flux was measured for five minutes before addition of ligand. Data in TABLE 8 represent the mean of the fluorescence ratio of a representative experiment, rounded to two decimal places. There
- 5 was no measurable activity of NR1/2A in the absence of ligand after addition of buffer only. Inhibition of NMDAR by addition of MK801 revealed only a weak decrease of signal in the absence of ligand, suggesting a low basal activity of NR1/2A (probably caused by the carry-over of endogenous ligand from the cell culture media).

TABLE 8				
NMDAR-Mediated Calcium Flux in the Presence of MK801				
time (sec)	DMSO + ligand (F/F ₀)	DMSO no ligand (F/F ₀)	MK801 (10 μ M) + ligand (F/F ₀)	MK801 (1 μ M) + ligand (F/F ₀)
0	1.00	1.00	1.00	1.00
1	1.02	1.01	1.00	0.99
2	0.98	1.00	0.98	1.00
3	1.00	1.01	0.98	0.98
4	0.99	1.01	0.99	0.97
5	1.00	0.98	0.99	0.98
6	1.01	1.00	1.00	0.99
7	0.98	1.01	1.00	0.96
8	0.98	0.99	0.99	0.98
9	0.99	1.00	0.99	0.98
10	0.98	0.99	0.99	0.98
11	0.99	0.99	0.98	0.96
12	1.01	1.01	0.97	0.97
13	1.00	0.98	0.98	0.96
14	0.98	0.98	0.98	0.97
15	0.98	1.01	1.00	0.97
16	0.97	1.00	0.97	0.98
17	0.99	0.97	0.98	0.98
18	0.96	0.99	0.98	0.97
19	0.97	0.99	0.97	0.96
20	0.97	0.97	0.94	0.94
21	0.96	0.99	0.95	0.93
22	0.96	1.00	0.95	0.96
23	0.94	1.02	0.95	0.95
24	0.97	1.03	0.95	0.97
25	0.95	1.02	0.98	0.98
26	0.96	1.02	0.98	0.96
27	0.96	1.01	0.95	0.96
28	0.96	1.01	0.94	0.95
29	0.96	1.01	0.96	0.97

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
30	0.96	1.02	0.93	0.95
31	0.96	1.03	0.96	0.96
32	0.95	1.01	0.97	0.96
33	0.96	1.05	0.95	0.97
34	0.96	1.03	0.95	0.96
35	0.98	1.00	0.94	0.94
36	0.98	0.99	0.94	0.96
37	0.98	1.02	0.97	0.99
38	0.97	1.02	0.95	0.94
39	0.96	1.02	0.95	0.95
40	0.97	1.00	0.96	0.94
41	0.95	1.03	0.94	0.94
42	0.97	1.02	0.95	0.93
43	0.96	1.02	0.94	0.96
44	0.96	1.01	0.95	0.95
45	0.97	1.02	0.94	0.97
46	0.95	1.03	0.94	0.93
47	1.01	1.03	0.94	0.94
48	0.97	1.02	0.94	0.94
49	0.98	0.99	0.94	0.93
50	0.98	1.02	0.96	0.94
51	0.99	1.01	0.93	0.95
52	0.96	1.00	0.95	0.95
53	0.96	1.02	0.94	0.94
54	0.98	1.02	0.93	0.94
55	0.95	1.02	0.93	0.93
56	0.98	1.00	0.93	0.93
57	0.99	1.01	0.94	0.94
58	0.98	1.01	0.95	0.92
59	0.95	1.03	0.93	0.94
60	0.94	1.02	0.95	0.96
61	0.97	1.02	0.92	0.91
62	0.97	1.01	0.93	0.92
63	0.96	1.01	0.94	0.93
64	0.97	1.04	0.95	0.96
65	0.99	1.03	0.93	0.93
66	0.96	1.01	0.94	0.93
67	0.99	1.00	0.94	0.94
68	0.97	1.01	0.92	0.95
69	0.96	1.04	0.94	0.93
70	0.96	1.02	0.94	0.92
71	0.96	1.01	0.93	0.93
72	0.97	1.00	0.93	0.92
73	0.96	1.02	0.94	0.94
74	0.97	1.03	0.93	0.92
75	0.97	1.02	0.94	0.92
76	0.96	1.01	0.93	0.92
77	0.96	1.00	0.94	0.93
78	0.95	1.00	0.90	0.93
79	0.97	1.05	0.93	0.92
80	0.96	0.99	0.92	0.91
81	0.98	1.01	0.93	0.92
82	0.98	1.00	0.94	0.93

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
83	0.96	1.04	0.94	0.93
84	0.97	1.01	0.91	0.93
85	0.96	1.04	0.94	0.94
86	0.96	1.03	0.92	0.91
87	0.97	1.00	0.92	0.93
88	0.96	1.02	0.93	0.91
89	0.96	1.01	0.92	0.92
90	0.97	1.01	0.91	0.93
91	0.98	1.00	0.92	0.90
92	0.97	1.02	0.91	0.92
93	0.96	1.00	0.91	0.92
94	1.00	1.01	0.93	0.94
95	0.98	1.00	0.90	0.92
96	0.98	1.00	0.93	0.89
97	0.97	1.02	0.91	0.92
98	0.96	1.03	0.91	0.92
99	0.98	1.01	0.92	0.95
100	0.98	1.02	0.91	0.92
101	0.97	1.02	0.92	0.89
102	0.96	1.01	0.89	0.92
103	0.97	1.01	0.91	0.90
104	0.98	1.03	0.93	0.90
105	0.96	1.03	0.92	0.92
106	0.98	1.02	0.91	0.89
107	0.96	1.01	0.91	0.93
108	0.96	1.04	0.92	0.92
109	0.97	1.01	0.92	0.92
110	0.97	1.04	0.91	0.91
111	0.99	1.03	0.92	0.90
112	0.97	0.99	0.92	0.92
113	0.98	1.01	0.91	0.90
114	0.97	1.03	0.92	0.92
115	0.97	1.00	0.91	0.90
116	0.98	1.02	0.91	0.92
117	0.96	1.01	0.90	0.93
118	0.98	1.00	0.92	0.92
119	0.98	1.04	0.90	0.91
120	0.95	1.04	0.90	0.91
121	0.96	1.02	0.92	0.92
122	0.98	1.01	0.87	0.90
123	0.97	1.01	0.92	0.90
124	0.97	1.00	0.89	0.88
125	0.96	1.00	0.91	0.91
126	0.95	1.01	0.91	0.91
127	0.95	1.02	0.90	0.92
128	0.99	1.02	0.90	0.90
129	0.96	1.02	0.89	0.91
130	0.94	1.00	0.91	0.92
131	0.94	0.99	0.91	0.90
132	0.97	1.03	0.91	0.88
133	0.96	1.03	0.91	0.92
134	0.97	1.01	0.91	0.91
135	0.97	1.02	0.91	0.90

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
136	0.98	0.98	0.91	0.90
137	0.96	1.00	0.91	0.90
138	0.94	1.01	0.89	0.90
139	0.96	1.03	0.92	0.90
140	0.97	1.01	0.92	0.91
141	0.96	1.01	0.88	0.90
142	0.97	1.03	0.91	0.90
143	0.96	1.01	0.93	0.90
144	0.97	1.01	0.90	0.91
145	0.98	1.02	0.91	0.89
146	0.96	1.01	0.90	0.90
147	0.97	1.02	0.89	0.90
148	0.96	1.00	0.90	0.91
149	0.95	1.01	0.90	0.90
150	0.98	1.00	0.89	0.91
151	0.95	1.00	0.90	0.91
152	0.96	0.99	0.91	0.91
153	0.96	1.03	0.90	0.90
154	0.95	1.02	0.90	0.89
155	0.97	1.00	0.89	0.90
156	0.96	1.01	0.89	0.91
157	0.96	1.02	0.91	0.90
158	0.93	1.00	0.91	0.90
159	0.94	1.00	0.90	0.91
160	0.97	1.03	0.90	0.90
161	0.94	1.00	0.90	0.92
162	0.97	1.00	0.88	0.89
163	0.97	1.05	0.90	0.89
164	0.95	1.00	0.90	0.91
165	0.96	1.02	0.90	0.90
166	0.98	1.02	0.90	0.89
167	0.96	1.02	0.91	0.90
168	0.97	1.00	0.90	0.90
169	0.95	1.00	0.89	0.90
170	0.97	1.01	0.89	0.90
171	0.98	1.02	0.90	0.90
172	0.96	1.00	0.90	0.91
173	0.97	0.99	0.90	0.91
174	0.96	0.99	0.90	0.88
175	0.95	1.02	0.90	0.90
176	0.94	1.00	0.90	0.89
177	0.99	1.04	0.90	0.91
178	0.95	1.00	0.90	0.91
179	0.93	1.00	0.89	0.90
180	0.96	1.00	0.88	0.90
181	0.96	0.99	0.92	0.90
182	0.94	1.02	0.90	0.90
183	0.95	0.99	0.90	0.87
184	0.96	1.00	0.91	0.89
185	0.95	1.01	0.91	0.90
186	0.94	1.01	0.91	0.89
187	0.96	1.04	0.90	0.91
188	0.95	1.01	0.89	0.89

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
189	0.96	1.02	0.91	0.89
190	0.96	1.00	0.91	0.88
191	0.95	1.03	0.89	0.91
192	0.95	1.00	0.91	0.90
193	0.97	1.01	0.88	0.89
194	0.94	1.00	0.90	0.90
195	0.96	1.02	0.90	0.89
196	0.96	1.01	0.90	0.91
197	0.95	1.00	0.90	0.91
198	0.94	1.03	0.89	0.90
199	0.97	0.99	0.90	0.89
200	0.94	0.99	0.91	0.91
201	0.93	0.99	0.90	0.90
202	0.96	0.99	0.88	0.88
203	0.96	1.02	0.88	0.88
204	0.96	0.99	0.89	0.89
205	0.95	0.99	0.88	0.92
206	0.94	0.99	0.89	0.89
207	0.98	0.99	0.90	0.90
208	0.94	1.00	0.89	0.88
209	0.96	1.01	0.88	0.89
210	0.95	1.00	0.90	0.88
211	0.92	1.01	0.89	0.91
212	0.95	0.97	0.90	0.89
213	0.94	1.01	0.88	0.88
214	0.95	1.01	0.90	0.89
215	0.95	1.00	0.88	0.87
216	0.94	0.98	0.89	0.87
217	0.95	1.00	0.90	0.89
218	0.95	1.01	0.90	0.90
219	0.95	0.99	0.90	0.88
220	0.95	1.00	0.88	0.88
221	0.96	1.01	0.89	0.89
222	0.95	1.01	0.89	0.88
223	0.96	0.99	0.89	0.89
224	0.93	0.99	0.90	0.89
225	0.93	0.99	0.89	0.86
226	0.95	1.00	0.88	0.89
227	0.95	0.99	0.89	0.90
228	0.94	1.03	0.88	0.89
229	0.94	1.01	0.89	0.88
230	0.94	0.97	0.88	0.87
231	0.95	1.02	0.87	0.89
232	0.93	1.01	0.88	0.88
233	0.95	0.99	0.88	0.88
234	0.94	1.00	0.90	0.87
235	0.94	1.01	0.88	0.89
236	0.95	1.00	0.89	0.90
237	0.93	0.99	0.89	0.89
238	0.96	1.02	0.89	0.89
239	0.96	1.02	0.90	0.90
240	0.96	1.01	0.87	0.89
241	0.93	1.00	0.87	0.87

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
242	0.96	1.01	0.87	0.90
243	0.96	1.02	0.88	0.88
244	0.94	1.00	0.89	0.88
245	0.91	0.99	0.89	0.88
246	0.95	1.00	0.89	0.91
247	0.94	0.98	0.87	0.89
248	0.96	1.01	0.88	0.89
249	0.93	0.99	0.89	0.89
250	0.91	1.00	0.88	0.89
251	0.94	1.00	0.89	0.88
252	0.95	0.99	0.89	0.89
253	0.96	1.00	0.90	0.88
254	0.95	0.97	0.89	0.87
255	0.94	1.00	0.88	0.87
256	0.95	0.98	0.89	0.88
257	0.92	0.99	0.88	0.86
258	0.91	0.97	0.89	0.87
259	0.94	0.99	0.88	0.88
260	0.92	0.98	0.87	0.89
261	0.92	0.99	0.87	0.87
262	0.93	0.97	0.90	0.87
263	0.92	0.98	0.88	0.87
264	0.92	1.00	0.89	0.89
265	0.93	1.00	0.87	0.86
266	0.93	0.99	0.87	0.88
267	0.92	1.01	0.87	0.88
268	0.92	0.99	0.88	0.88
269	0.93	1.01	0.89	0.86
270	0.91	0.99	0.89	0.87
271	0.92	1.02	0.89	0.88
272	0.91	0.95	0.87	0.86
273	0.91	0.98	0.87	0.86
274	0.93	0.96	0.88	0.88
275	0.93	0.99	0.86	0.89
276	0.92	0.98	0.88	0.88
277	0.94	1.01	0.88	0.86
278	0.93	0.98	0.89	0.87
279	0.93	1.00	0.87	0.87
280	0.92	0.98	0.87	0.88
281	0.93	0.99	0.90	0.86
282	0.91	0.99	0.87	0.88
283	0.93	0.97	0.89	0.86
284	0.92	0.99	0.87	0.87
285	0.92	1.00	0.87	0.88
286	0.93	0.97	0.86	0.87
287	0.90	0.99	0.88	0.88
288	0.92	0.98	0.88	0.88
289	0.91	0.97	0.88	0.87
290	0.92	1.00	0.85	0.84
291	0.96	1.02	0.85	0.86
292	0.97	1.02	0.86	0.90
293	1.00	1.05	0.88	0.92
294	1.01	1.04	0.89	0.92

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
295	1.01	1.04	0.89	0.93
296	1.02	1.03	0.86	0.91
297	1.09	1.03	0.88	0.93
298	1.08	1.04	0.88	0.94
299	1.12	1.04	0.90	0.95
300	1.14	1.03	0.89	0.98
301	1.17	1.02	0.88	0.99
302	1.19	1.05	0.88	0.97
303	1.20	1.02	0.88	0.99
304	1.24	1.03	0.88	1.02
305	1.27	1.02	0.88	1.02
306	1.32	1.05	0.88	1.03
307	1.35	1.04	0.88	1.05
308	1.36	1.04	0.87	1.05
309	1.40	1.03	0.89	1.06
310	1.43	1.01	0.88	1.08
311	1.43	1.06	0.88	1.09
312	1.48	1.04	0.88	1.10
313	1.51	1.02	0.88	1.09
314	1.53	1.03	0.86	1.11
315	1.55	1.03	0.88	1.13
316	1.57	1.01	0.87	1.12
317	1.62	1.03	0.87	1.14
318	1.62	1.02	0.88	1.13
319	1.64	1.02	0.89	1.13
320	1.65	1.02	0.87	1.16
321	1.69	1.02	0.87	1.18
322	1.73	1.00	0.88	1.16
323	1.71	1.03	0.88	1.15
324	1.75	1.03	0.89	1.15
325	1.74	1.04	0.87	1.16
326	1.76	1.02	0.87	1.17
327	1.79	1.03	0.87	1.16
328	1.82	1.02	0.88	1.16
329	1.85	1.00	0.88	1.21
330	1.84	1.05	0.86	1.19
331	1.87	1.01	0.88	1.20
332	1.88	1.04	0.88	1.18
333	1.87	1.04	0.88	1.23
334	1.89	1.02	0.88	1.21
335	1.91	1.00	0.87	1.20
336	1.91	1.02	0.87	1.19
337	1.94	1.03	0.89	1.21
338	1.94	1.02	0.86	1.19
339	1.96	1.00	0.86	1.20
340	1.98	1.01	0.86	1.20
341	1.99	1.00	0.87	1.18
342	2.01	1.04	0.87	1.20
343	1.98	1.01	0.86	1.17
344	2.03	1.01	0.88	1.19
345	2.02	1.03	0.88	1.18
346	2.02	1.00	0.87	1.20
347	2.05	1.04	0.88	1.18

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
348	2.08	1.02	0.86	1.19
349	2.09	1.01	0.86	1.19
350	2.08	1.04	0.86	1.19
351	2.09	1.02	0.87	1.19
352	2.09	1.01	0.86	1.17
353	2.12	1.01	0.87	1.17
354	2.12	1.02	0.86	1.18
355	2.15	1.03	0.86	1.15
356	2.14	1.02	0.86	1.17
357	2.16	1.02	0.85	1.16
358	2.15	1.04	0.88	1.16
359	2.15	1.03	0.88	1.14
360	2.15	1.02	0.85	1.17
361	2.18	1.00	0.86	1.16
362	2.20	1.02	0.84	1.17
363	2.20	1.02	0.87	1.18
364	2.19	1.00	0.85	1.16
365	2.20	1.00	0.85	1.14
366	2.22	1.01	0.88	1.15
367	2.23	1.03	0.85	1.14
368	2.22	1.04	0.85	1.15
369	2.24	1.02	0.85	1.13
370	2.23	1.00	0.84	1.13
371	2.25	0.99	0.86	1.13
372	2.25	1.02	0.85	1.14
373	2.26	1.03	0.84	1.11
374	2.27	1.01	0.85	1.14
375	2.27	1.01	0.84	1.10
376	2.27	1.02	0.86	1.12
377	2.26	1.03	0.86	1.09
378	2.28	1.01	0.86	1.12
379	2.29	1.00	0.85	1.11
380	2.27	1.03	0.86	1.09
381	2.33	1.03	0.87	1.12
382	2.28	0.99	0.85	1.11
383	2.31	1.04	0.85	1.08
384	2.33	1.01	0.87	1.11
385	2.32	1.02	0.84	1.09
386	2.33	1.01	0.86	1.11
387	2.31	1.02	0.85	1.08
388	2.32	1.04	0.85	1.08
389	2.33	1.02	0.84	1.08
390	2.32	1.01	0.85	1.06
391	2.34	1.02	0.85	1.10
392	2.35	1.02	0.87	1.08
393	2.34	1.02	0.86	1.07
394	2.38	1.01	0.85	1.08
395	2.34	1.01	0.85	1.08
396	2.37	1.01	0.84	1.07
397	2.36	1.02	0.83	1.05
398	2.37	1.04	0.86	1.06
399	2.40	1.02	0.85	1.05
400	2.36	1.02	0.83	1.04

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
401	2.36	1.01	0.84	1.07
402	2.38	1.00	0.84	1.04
403	2.39	1.01	0.84	1.05
404	2.39	1.02	0.85	1.05
405	2.40	1.02	0.85	1.05
406	2.39	0.99	0.85	1.03
407	2.38	0.99	0.83	1.05
408	2.41	1.03	0.85	1.05
409	2.43	1.00	0.83	1.04
410	2.41	1.03	0.84	1.04
411	2.42	1.01	0.83	1.03
412	2.43	1.03	0.84	1.04
413	2.41	1.00	0.84	1.02
414	2.42	1.01	0.85	1.03
415	2.42	1.00	0.84	1.04
416	2.42	1.01	0.84	1.06
417	2.44	1.00	0.83	1.03
418	2.44	0.99	0.84	1.02
419	2.43	1.01	0.83	1.02
420	2.43	0.99	0.82	1.03
421	2.45	1.01	0.85	1.01
422	2.45	1.02	0.83	1.01
423	2.43	1.00	0.81	1.01
424	2.45	1.02	0.82	1.02
425	2.44	1.02	0.82	1.00
426	2.42	1.01	0.81	1.02
427	2.44	0.99	0.81	1.02
428	2.46	1.02	0.83	1.01
429	2.45	1.02	0.83	1.01
430	2.45	1.02	0.84	1.02
431	2.43	1.01	0.84	1.03
432	2.42	1.03	0.83	1.02
433	2.46	1.00	0.84	1.00
434	2.47	1.01	0.83	1.00
435	2.46	1.01	0.84	1.01
436	2.47	1.01	0.82	1.02
437	2.45	1.01	0.84	1.00
438	2.48	1.02	0.82	1.01
439	2.45	0.99	0.81	1.00
440	2.46	1.00	0.84	1.01
441	2.45	1.01	0.82	1.00
442	2.46	1.01	0.83	1.01
443	2.46	1.00	0.83	1.00
444	2.49	1.00	0.82	1.00
445	2.46	0.99	0.83	0.99
446	2.46	1.00	0.83	0.99
447	2.47	1.01	0.83	0.98
448	2.46	1.01	0.82	0.99
449	2.48	1.00	0.81	0.98
450	2.51	0.99	0.84	0.98
451	2.50	1.00	0.84	0.98
452	2.47	1.00	0.83	0.98
453	2.48	1.01	0.81	0.98

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
454	2.49	1.03	0.83	0.98
455	2.49	1.02	0.81	0.96
456	2.49	1.00	0.82	0.99
457	2.46	1.00	0.83	0.98
458	2.45	1.00	0.83	0.98
459	2.48	1.01	0.84	0.98
460	2.48	1.00	0.82	0.99
461	2.47	0.98	0.82	0.99
462	2.49	1.03	0.84	0.99
463	2.48	0.99	0.82	0.96
464	2.50	1.00	0.81	0.98
465	2.47	1.00	0.83	0.96
466	2.45	0.99	0.82	0.98
467	2.50	1.03	0.82	0.97
468	2.47	1.01	0.84	0.97
469	2.48	0.99	0.82	0.97
470	2.45	1.02	0.81	0.97
471	2.45	1.02	0.82	0.98
472	2.50	1.01	0.82	0.98
473	2.48	1.02	0.81	0.96
474	2.46	1.02	0.81	0.96
475	2.49	1.01	0.83	0.98
476	2.46	1.01	0.82	0.98
477	2.45	0.98	0.81	0.96
478	2.47	1.00	0.81	0.97
479	2.50	1.02	0.81	0.96
480	2.44	1.00	0.83	0.97
481	2.47	0.99	0.82	0.97
482	2.47	1.00	0.82	0.96
483	2.44	0.99	0.81	0.97
484	2.46	0.97	0.83	0.95
485	2.48	1.00	0.82	0.96
486	2.47	0.99	0.83	0.95
487	2.48	1.00	0.82	0.96
488	2.50	0.99	0.83	0.95
489	2.47	1.00	0.82	0.95
490	2.45	0.98	0.81	0.96
491	2.46	1.02	0.82	0.96
492	2.47	0.98	0.82	0.96
493	2.48	0.99	0.82	0.96
494	2.49	0.98	0.82	0.95
495	2.49	0.99	0.83	0.94
496	2.47	1.01	0.81	0.93
497	2.45	0.99	0.82	0.94
498	2.48	1.01	0.84	0.94
499	2.45	0.98	0.82	0.93
500	2.49	0.98	0.84	0.95
501	2.45	0.99	0.82	0.95
502	2.47	0.97	0.83	0.96
503	2.46	1.01	0.83	0.94
504	2.46	0.98	0.82	0.95
505	2.44	1.01	0.83	0.95
506	2.47	1.00	0.82	0.95

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
507	2.44	0.98	0.82	0.95
508	2.45	1.00	0.83	0.94
509	2.43	0.99	0.82	0.94
510	2.47	0.99	0.83	0.94
511	2.45	1.03	0.83	0.94
512	2.48	0.98	0.82	0.94
513	2.47	0.97	0.81	0.97
514	2.46	0.97	0.80	0.93
515	2.45	1.00	0.82	0.94
516	2.44	1.00	0.81	0.93
517	2.43	0.99	0.81	0.92
518	2.45	0.99	0.81	0.93
519	2.45	0.97	0.82	0.93
520	2.44	1.01	0.81	0.93
521	2.47	0.99	0.82	0.93
522	2.42	0.99	0.81	0.92
523	2.46	1.00	0.83	0.94
524	2.43	1.00	0.81	0.94
525	2.45	1.00	0.82	0.93
526	2.43	1.00	0.82	0.95
527	2.43	0.97	0.82	0.93
528	2.46	0.99	0.83	0.92
529	2.44	1.00	0.82	0.94
530	2.42	0.99	0.81	0.93
531	2.43	0.99	0.82	0.93
532	2.42	0.97	0.81	0.94
533	2.42	0.99	0.80	0.95
534	2.42	0.99	0.82	0.92
535	2.40	0.98	0.82	0.92
536	2.43	0.98	0.81	0.93
537	2.42	0.98	0.81	0.93
538	2.40	0.99	0.81	0.93
539	2.42	0.99	0.81	0.91
540	2.42	0.99	0.80	0.94
541	2.44	1.01	0.82	0.92
542	2.41	0.99	0.82	0.92
543	2.42	0.99	0.82	0.93
544	2.42	1.00	0.82	0.91
545	2.41	0.98	0.82	0.92
546	2.42	0.97	0.80	0.94
547	2.40	0.97	0.83	0.92
548	2.43	0.99	0.82	0.94
549	2.40	1.00	0.82	0.93
550	2.44	0.98	0.83	0.92
551	2.41	0.98	0.82	0.94
552	2.40	1.00	0.82	0.92
553	2.41	0.99	0.81	0.94
554	2.40	0.98	0.81	0.92
555	2.39	0.98	0.81	0.92
556	2.40	0.99	0.82	0.92
557	2.38	0.97	0.79	0.93
558	2.39	0.97	0.81	0.91
559	2.37	0.98	0.81	0.94

TABLE 8
NMDAR-Mediated Calcium Flux in the Presence of MK801

<u>time (sec)</u>	<u>DMSO</u> <u>+ ligand (F/F₀)</u>	<u>DMSO</u> <u>no ligand (F/F₀)</u>	<u>MK801 (10 μM)</u> <u>+ ligand (F/F₀)</u>	<u>MK801 (1 μM)</u> <u>+ ligand (F/F₀)</u>
560	2.38	0.97	0.83	0.93
561	2.40	0.98	0.83	0.93
562	2.40	0.98	0.82	0.93
563	2.38	0.98	0.81	0.91
564	2.37	0.97	0.80	0.92
565	2.38	0.98	0.79	0.93
566	2.37	0.98	0.82	0.90
567	2.39	0.97	0.81	0.91
568	2.37	0.96	0.82	0.92
569	2.39	0.97	0.82	0.92
570	2.36	0.98	0.81	0.92
571	2.34	0.97	0.82	0.93
572	2.37	0.99	0.82	0.93
573	2.35	0.98	0.80	0.91
574	2.35	0.99	0.78	0.92
575	2.39	0.96	0.81	0.92
576	2.34	0.96	0.81	0.91
577	2.37	0.99	0.80	0.93
578	2.37	0.97	0.80	0.92
579	2.35	0.95	0.82	0.91
580	2.36	0.96	0.81	0.93
581	2.37	0.97	0.81	0.92
582	2.35	0.97	0.82	0.91
583	2.34	0.96	0.81	0.91
584	2.34	1.00	0.81	0.92
585	2.32	0.97	0.80	0.91
586	2.33	0.95	0.81	0.93
587	2.34	0.96	0.80	0.91
588	2.36	0.95	0.81	0.92
589	2.34	0.99	0.80	0.91
590	2.32	0.96	0.81	0.91
591	2.33	0.98	0.81	0.92
592	2.35	0.97	0.81	0.89
593	2.32	0.93	0.82	0.92
594	2.35	0.97	0.81	0.90
595	2.31	0.99	0.80	0.91
596	2.32	0.97	0.82	0.93
597	2.33	0.97	0.80	0.91
598	2.32	0.96	0.81	0.91
599	2.31	0.96	0.80	0.91

[097] The ligand-independent activity was much weaker than observed in previous expression systems for NMDAR, likely because of the high expression of NMDAR and the high levels of glutamate and or glycine released into the media in these cells.

- 5 **[098]** Taken together, our results demonstrate baculovirus mediated expression of NMDAR in HEK293 cells in the presence of MDL105,519 as an improved method to measure NMDAR activity in non-neuronal cells.

[099] Other subunits of NMDAR. Having introduced baculovirus mediated expression of NR1/2A in combination with protection of the cells using MDL105,519 as a novel assay

system for the study of NMDAR-activity, we wondered whether the same system could be used for other subunits of NMDAR. To test this hypothesis, we transduced HEK293 cells with varying amounts of NR1 and NR2B (GRIN2B) in the presence of MDL105,519 and measured NMDAR-mediated calcium flux on the FDSS.

- 5 **[0100]** As with NR1/2A, we could detect NMDAR-mediated calcium flux after stimulation with glutamate and glycine in a concentration dependent manner. See, TABLE 9, showing the ligand concentration dependency of NR1/2B-mediated calcium flux. HEK293 cells were transduced with NR1/2B baculovirus in the presence of MDL105,519, loaded with calcium 6 dye and NMDAR-mediated calcium flux was measured after stimulation of
- 10 the indicated concentration of both ligands. Data in TABLE 9 represent the mean of the fluorescence ratio of a representative experiment, rounded to two decimal places.

TABLE 9 Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux									
time sec	100.00 μ M	50.00 μ M	25.00 μ M	12.50 μ M	6.25 μ M	3.13 μ M	1.67 μ M	0.84 μ M	no ligand
0.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.0	1.00	1.00	1.01	1.00	1.00	1.01	1.00	1.00	1.00
0.5	1.00	1.00	1.00	1.00	1.00	1.01	1.00	1.00	1.00
1.0	1.00	1.00	1.00	1.00	1.00	1.01	1.00	1.00	1.00
1.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.5	0.99	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00
3.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4.0	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.0	0.99	0.99	0.98	0.98	0.98	0.99	0.98	0.99	0.99
5.5	0.97	0.98	0.98	0.98	0.97	0.98	0.98	0.98	0.99
6.0	0.97	0.98	0.99	0.98	0.98	0.98	0.98	0.98	1.00
6.5	0.97	0.98	0.98	0.97	0.97	0.97	0.97	0.98	0.99
7.0	0.97	0.98	0.98	0.98	0.98	0.98	0.98	0.98	1.00
7.5	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	1.00
8.0	0.98	0.98	0.99	0.99	0.98	0.98	0.99	0.99	1.00
8.5	0.98	0.99	0.99	0.99	0.98	0.98	0.98	0.99	1.00
9.0	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	1.01
9.5	0.99	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00
10.0	1.00	0.99	1.00	1.00	0.99	0.99	0.99	1.00	1.01
10.5	1.00	0.99	0.99	0.99	0.99	0.99	0.99	1.00	1.01
11.0	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.01
11.5	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.01
12.0	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.01
12.5	1.00	1.01	1.01	1.01	1.00	1.00	1.00	1.01	1.02
13.0	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.01	1.02
13.5	1.01	1.01	1.01	1.01	1.00	1.00	1.01	1.01	1.02
14.0	1.01	1.01	1.01	1.01	1.01	1.00	1.01	1.01	1.02
14.5	1.02	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.02
15.0	1.01	1.01	1.01	1.01	1.00	1.01	1.01	1.01	1.02
15.5	1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.01	1.03
16.0	1.02	1.02	1.02	1.02	1.01	1.01	1.02	1.02	1.02
16.5	1.03	1.02	1.02	1.02	1.01	1.01	1.01	1.02	1.03
17.0	1.02	1.02	1.02	1.02	1.01	1.01	1.02	1.02	1.03

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
17.5	1.03	1.02	1.02	1.02	1.01	1.02	1.02	1.02	1.03
18.0	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.03
18.5	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.03
19.0	1.04	1.03	1.03	1.03	1.02	1.02	1.03	1.03	1.03
19.5	1.04	1.03	1.03	1.03	1.02	1.02	1.03	1.02	1.03
20.0	1.04	1.04	1.03	1.03	1.02	1.03	1.03	1.03	1.03
20.5	1.04	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.03
21.0	1.04	1.04	1.03	1.03	1.03	1.02	1.03	1.03	1.03
21.5	1.04	1.03	1.03	1.03	1.03	1.02	1.02	1.03	1.03
22.0	1.05	1.04	1.04	1.03	1.03	1.03	1.03	1.03	1.03
22.5	1.05	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03
23.0	1.05	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03
23.5	1.05	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03
24.0	1.05	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03
24.5	1.05	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03
25.0	1.06	1.04	1.05	1.05	1.03	1.03	1.04	1.04	1.03
25.5	1.06	1.05	1.05	1.05	1.03	1.03	1.04	1.04	1.04
26.0	1.06	1.05	1.05	1.04	1.03	1.03	1.04	1.03	1.04
26.5	1.06	1.05	1.05	1.04	1.03	1.03	1.04	1.04	1.03
27.0	1.07	1.05	1.05	1.04	1.04	1.03	1.04	1.04	1.04
27.5	1.07	1.05	1.05	1.05	1.04	1.03	1.04	1.04	1.04
28.0	1.07	1.05	1.05	1.05	1.04	1.03	1.04	1.04	1.04
28.5	1.07	1.06	1.05	1.05	1.04	1.04	1.04	1.04	1.04
29.0	1.07	1.06	1.05	1.05	1.04	1.04	1.04	1.04	1.04
29.5	1.08	1.06	1.06	1.06	1.04	1.04	1.04	1.05	1.04
30.0	1.08	1.06	1.06	1.05	1.04	1.04	1.05	1.04	1.04
30.5	1.08	1.06	1.06	1.06	1.05	1.04	1.05	1.05	1.04
31.0	1.08	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.04
31.5	1.08	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.04
32.0	1.09	1.06	1.06	1.06	1.05	1.04	1.05	1.05	1.04
32.5	1.09	1.06	1.07	1.07	1.05	1.04	1.05	1.05	1.04
33.0	1.09	1.07	1.07	1.06	1.05	1.05	1.05	1.05	1.04
33.5	1.09	1.07	1.07	1.07	1.06	1.05	1.05	1.05	1.04
34.0	1.09	1.07	1.06	1.06	1.05	1.05	1.05	1.05	1.04
34.5	1.09	1.07	1.07	1.07	1.06	1.05	1.05	1.05	1.04
35.0	1.09	1.07	1.07	1.07	1.06	1.05	1.06	1.05	1.05
35.5	1.10	1.07	1.07	1.06	1.05	1.05	1.05	1.05	1.04
36.0	1.10	1.07	1.07	1.07	1.05	1.05	1.06	1.05	1.04
36.5	1.10	1.08	1.07	1.07	1.06	1.05	1.06	1.06	1.04
37.0	1.11	1.08	1.08	1.07	1.06	1.06	1.06	1.05	1.05
37.5	1.11	1.08	1.08	1.07	1.06	1.05	1.06	1.06	1.05
38.0	1.11	1.08	1.08	1.08	1.06	1.06	1.06	1.05	1.04
38.5	1.11	1.08	1.08	1.07	1.06	1.05	1.06	1.05	1.04
39.0	1.11	1.09	1.08	1.08	1.06	1.06	1.06	1.06	1.04
39.5	1.11	1.09	1.08	1.08	1.06	1.06	1.06	1.06	1.04
40.0	1.12	1.09	1.08	1.07	1.06	1.05	1.06	1.05	1.04
40.5	1.12	1.09	1.09	1.08	1.07	1.06	1.06	1.06	1.04
41.0	1.12	1.09	1.09	1.08	1.07	1.06	1.06	1.06	1.04
41.5	1.12	1.09	1.09	1.08	1.07	1.06	1.06	1.06	1.04
42.0	1.12	1.09	1.09	1.08	1.07	1.06	1.07	1.06	1.05
42.5	1.13	1.09	1.09	1.08	1.07	1.06	1.07	1.06	1.05
43.0	1.12	1.09	1.09	1.08	1.07	1.06	1.06	1.06	1.04
43.5	1.13	1.10	1.09	1.09	1.07	1.06	1.07	1.06	1.05

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
44.0	1.13	1.10	1.09	1.09	1.07	1.06	1.07	1.06	1.05
44.5	1.13	1.10	1.10	1.09	1.07	1.06	1.06	1.06	1.04
45.0	1.13	1.10	1.09	1.08	1.07	1.06	1.07	1.06	1.04
45.5	1.13	1.10	1.09	1.09	1.07	1.06	1.07	1.06	1.04
46.0	1.14	1.10	1.10	1.09	1.07	1.06	1.07	1.06	1.05
46.5	1.14	1.11	1.10	1.09	1.07	1.06	1.07	1.07	1.05
47.0	1.14	1.10	1.10	1.09	1.07	1.06	1.07	1.06	1.04
47.5	1.14	1.11	1.10	1.09	1.08	1.06	1.07	1.06	1.05
48.0	1.14	1.11	1.10	1.09	1.08	1.06	1.07	1.07	1.04
48.5	1.14	1.11	1.10	1.09	1.07	1.06	1.07	1.06	1.04
49.0	1.15	1.11	1.11	1.10	1.08	1.06	1.07	1.07	1.05
49.5	1.15	1.11	1.10	1.09	1.08	1.06	1.07	1.06	1.04
50.0	1.15	1.11	1.11	1.10	1.08	1.06	1.07	1.07	1.05
50.5	1.16	1.11	1.11	1.10	1.08	1.07	1.08	1.07	1.05
51.0	1.15	1.11	1.11	1.10	1.08	1.07	1.07	1.07	1.05
51.5	1.15	1.12	1.11	1.10	1.08	1.07	1.07	1.07	1.05
52.0	1.16	1.12	1.11	1.10	1.08	1.07	1.07	1.07	1.05
52.5	1.16	1.12	1.11	1.10	1.08	1.07	1.08	1.07	1.05
53.0	1.16	1.12	1.11	1.10	1.08	1.07	1.08	1.07	1.05
53.5	1.16	1.12	1.11	1.10	1.08	1.07	1.08	1.06	1.05
54.0	1.17	1.12	1.11	1.11	1.08	1.07	1.08	1.07	1.05
54.5	1.17	1.12	1.11	1.10	1.08	1.07	1.08	1.07	1.05
55.0	1.17	1.12	1.12	1.11	1.08	1.07	1.08	1.07	1.05
55.5	1.17	1.12	1.12	1.10	1.08	1.07	1.08	1.07	1.04
56.0	1.17	1.13	1.12	1.11	1.09	1.07	1.08	1.07	1.05
56.5	1.18	1.13	1.12	1.11	1.09	1.08	1.08	1.07	1.05
57.0	1.18	1.13	1.12	1.11	1.09	1.08	1.08	1.07	1.05
57.5	1.18	1.13	1.12	1.11	1.09	1.07	1.08	1.07	1.05
58.0	1.18	1.13	1.13	1.11	1.09	1.07	1.08	1.07	1.05
58.5	1.18	1.13	1.13	1.11	1.09	1.07	1.08	1.07	1.05
59.0	1.18	1.13	1.13	1.12	1.09	1.08	1.08	1.07	1.05
59.5	1.18	1.13	1.12	1.11	1.09	1.07	1.08	1.07	1.05
60.0	1.18	1.13	1.13	1.11	1.09	1.08	1.08	1.07	1.05
60.5	1.18	1.13	1.13	1.11	1.09	1.07	1.08	1.07	1.05
61.0	1.19	1.13	1.13	1.12	1.09	1.08	1.08	1.08	1.05
61.5	1.19	1.14	1.13	1.12	1.09	1.08	1.08	1.07	1.05
62.0	1.19	1.13	1.13	1.12	1.09	1.08	1.08	1.07	1.05
62.5	1.18	1.14	1.13	1.12	1.09	1.08	1.08	1.07	1.05
63.0	1.19	1.14	1.13	1.12	1.09	1.08	1.09	1.07	1.05
63.5	1.19	1.14	1.13	1.12	1.10	1.08	1.08	1.07	1.05
64.0	1.19	1.14	1.13	1.12	1.09	1.08	1.08	1.07	1.05
64.5	1.20	1.14	1.13	1.12	1.09	1.08	1.08	1.08	1.05
65.0	1.20	1.14	1.14	1.12	1.10	1.08	1.08	1.08	1.05
65.5	1.20	1.14	1.14	1.12	1.09	1.08	1.08	1.08	1.05
66.0	1.20	1.15	1.14	1.12	1.10	1.08	1.09	1.08	1.05
66.5	1.20	1.15	1.14	1.12	1.10	1.08	1.09	1.08	1.05
67.0	1.20	1.15	1.14	1.13	1.10	1.08	1.08	1.07	1.05
67.5	1.20	1.15	1.14	1.13	1.10	1.09	1.09	1.08	1.05
68.0	1.20	1.15	1.14	1.12	1.10	1.08	1.08	1.07	1.05
68.5	1.21	1.15	1.15	1.13	1.10	1.08	1.09	1.08	1.05
69.0	1.20	1.15	1.14	1.12	1.10	1.08	1.09	1.08	1.05
69.5	1.20	1.15	1.14	1.13	1.10	1.08	1.09	1.08	1.05
70.0	1.21	1.15	1.15	1.13	1.10	1.08	1.09	1.08	1.05

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
70.5	1.21	1.15	1.15	1.13	1.10	1.08	1.09	1.08	1.05
71.0	1.21	1.15	1.15	1.13	1.10	1.08	1.09	1.08	1.05
71.5	1.21	1.16	1.15	1.13	1.10	1.08	1.09	1.08	1.05
72.0	1.22	1.16	1.15	1.13	1.10	1.08	1.09	1.08	1.04
72.5	1.22	1.16	1.15	1.13	1.10	1.08	1.09	1.08	1.05
73.0	1.22	1.16	1.15	1.13	1.11	1.08	1.09	1.08	1.05
73.5	1.22	1.16	1.15	1.13	1.10	1.08	1.09	1.08	1.05
74.0	1.22	1.16	1.15	1.13	1.10	1.09	1.09	1.08	1.04
74.5	1.22	1.16	1.15	1.13	1.11	1.08	1.09	1.07	1.05
75.0	1.22	1.17	1.15	1.14	1.11	1.08	1.09	1.08	1.05
75.5	1.23	1.17	1.15	1.13	1.10	1.09	1.09	1.08	1.05
76.0	1.23	1.17	1.15	1.14	1.11	1.09	1.09	1.08	1.05
76.5	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.05
77.0	1.23	1.17	1.15	1.14	1.11	1.09	1.09	1.08	1.05
77.5	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.04
78.0	1.23	1.17	1.16	1.14	1.11	1.09	1.10	1.08	1.05
78.5	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.05
79.0	1.24	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.05
79.5	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.04
80.0	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.04
80.5	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.05
81.0	1.23	1.17	1.16	1.14	1.11	1.09	1.09	1.08	1.04
81.5	1.24	1.18	1.16	1.14	1.11	1.09	1.10	1.08	1.05
82.0	1.24	1.18	1.16	1.14	1.11	1.09	1.10	1.08	1.04
82.5	1.24	1.18	1.17	1.14	1.11	1.09	1.09	1.08	1.05
83.0	1.24	1.18	1.17	1.15	1.11	1.09	1.09	1.08	1.05
83.5	1.25	1.18	1.16	1.14	1.11	1.09	1.10	1.08	1.05
84.0	1.24	1.18	1.16	1.15	1.11	1.09	1.10	1.08	1.05
84.5	1.25	1.18	1.17	1.15	1.12	1.09	1.10	1.08	1.05
85.0	1.24	1.18	1.17	1.15	1.11	1.09	1.10	1.08	1.05
85.5	1.25	1.18	1.17	1.15	1.11	1.09	1.10	1.08	1.05
86.0	1.25	1.18	1.17	1.15	1.11	1.09	1.09	1.08	1.05
86.5	1.25	1.18	1.17	1.15	1.12	1.09	1.10	1.08	1.05
87.0	1.25	1.19	1.17	1.15	1.11	1.09	1.10	1.08	1.05
87.5	1.25	1.18	1.16	1.15	1.11	1.09	1.09	1.08	1.05
88.0	1.25	1.18	1.17	1.15	1.12	1.09	1.09	1.08	1.05
88.5	1.26	1.19	1.17	1.15	1.11	1.09	1.10	1.08	1.04
89.0	1.26	1.19	1.17	1.15	1.12	1.09	1.10	1.08	1.05
89.5	1.26	1.19	1.17	1.15	1.12	1.09	1.10	1.08	1.05
90.0	1.26	1.19	1.17	1.16	1.12	1.10	1.10	1.09	1.04
90.5	1.26	1.19	1.17	1.15	1.12	1.09	1.10	1.08	1.05
91.0	1.26	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.05
91.5	1.26	1.19	1.17	1.15	1.12	1.09	1.10	1.08	1.05
92.0	1.26	1.19	1.17	1.15	1.11	1.09	1.10	1.08	1.05
92.5	1.26	1.19	1.17	1.16	1.11	1.09	1.10	1.08	1.04
93.0	1.27	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.05
93.5	1.27	1.20	1.18	1.15	1.11	1.09	1.10	1.08	1.04
94.0	1.26	1.20	1.18	1.15	1.12	1.09	1.10	1.08	1.04
94.5	1.26	1.20	1.18	1.16	1.12	1.09	1.10	1.09	1.04
95.0	1.26	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.05
95.5	1.27	1.20	1.18	1.16	1.12	1.10	1.10	1.09	1.05
96.0	1.27	1.20	1.18	1.16	1.12	1.09	1.10	1.09	1.05
96.5	1.27	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.04

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
97.0	1.27	1.20	1.18	1.16	1.12	1.10	1.10	1.09	1.05
97.5	1.27	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.04
98.0	1.28	1.20	1.19	1.16	1.12	1.10	1.10	1.09	1.05
98.5	1.28	1.21	1.18	1.16	1.12	1.10	1.10	1.09	1.05
99.0	1.28	1.21	1.19	1.16	1.12	1.10	1.10	1.09	1.05
99.5	1.27	1.20	1.18	1.16	1.12	1.09	1.10	1.08	1.05
100.0	1.27	1.21	1.19	1.16	1.12	1.10	1.10	1.09	1.05
100.5	1.28	1.21	1.19	1.17	1.12	1.10	1.10	1.08	1.05
101.0	1.28	1.21	1.19	1.16	1.12	1.09	1.10	1.08	1.05
101.5	1.28	1.21	1.18	1.16	1.12	1.10	1.10	1.09	1.05
102.0	1.28	1.21	1.19	1.17	1.12	1.10	1.10	1.09	1.05
102.5	1.29	1.21	1.19	1.16	1.13	1.10	1.11	1.09	1.05
103.0	1.28	1.21	1.19	1.17	1.12	1.10	1.10	1.09	1.05
103.5	1.28	1.21	1.19	1.17	1.12	1.10	1.10	1.09	1.05
104.0	1.28	1.21	1.20	1.17	1.12	1.10	1.11	1.09	1.05
104.5	1.28	1.21	1.19	1.16	1.12	1.10	1.10	1.09	1.05
105.0	1.28	1.21	1.19	1.17	1.12	1.10	1.10	1.08	1.05
105.5	1.28	1.22	1.19	1.16	1.12	1.10	1.10	1.09	1.04
106.0	1.28	1.21	1.19	1.16	1.12	1.10	1.10	1.08	1.04
106.5	1.28	1.22	1.20	1.17	1.13	1.10	1.10	1.09	1.05
107.0	1.29	1.21	1.19	1.17	1.13	1.10	1.10	1.08	1.04
107.5	1.30	1.22	1.20	1.17	1.13	1.10	1.11	1.09	1.05
108.0	1.29	1.21	1.19	1.17	1.13	1.10	1.10	1.09	1.04
108.5	1.29	1.21	1.19	1.17	1.13	1.10	1.10	1.09	1.05
109.0	1.30	1.22	1.20	1.17	1.13	1.10	1.10	1.09	1.05
109.5	1.29	1.21	1.19	1.17	1.12	1.09	1.10	1.08	1.04
110.0	1.30	1.22	1.20	1.17	1.12	1.10	1.10	1.09	1.04
110.5	1.29	1.21	1.20	1.17	1.12	1.09	1.10	1.09	1.04
111.0	1.30	1.22	1.20	1.17	1.13	1.10	1.10	1.09	1.04
111.5	1.29	1.22	1.20	1.17	1.12	1.10	1.10	1.08	1.04
112.0	1.30	1.22	1.20	1.17	1.12	1.10	1.10	1.09	1.04
112.5	1.29	1.22	1.20	1.17	1.12	1.10	1.10	1.08	1.04
113.0	1.30	1.23	1.20	1.17	1.13	1.10	1.10	1.09	1.04
113.5	1.30	1.23	1.20	1.17	1.13	1.10	1.10	1.08	1.04
114.0	1.30	1.23	1.20	1.17	1.13	1.10	1.10	1.08	1.04
114.5	1.30	1.22	1.20	1.17	1.12	1.10	1.10	1.09	1.04
115.0	1.30	1.22	1.20	1.17	1.13	1.10	1.10	1.08	1.04
115.5	1.30	1.23	1.20	1.17	1.13	1.10	1.10	1.09	1.04
116.0	1.30	1.22	1.20	1.17	1.12	1.10	1.10	1.08	1.04
116.5	1.30	1.22	1.20	1.17	1.13	1.10	1.10	1.08	1.04
117.0	1.30	1.22	1.20	1.18	1.13	1.10	1.10	1.08	1.04
117.5	1.30	1.23	1.21	1.18	1.13	1.10	1.10	1.09	1.04
118.0	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.09	1.04
118.5	1.31	1.23	1.21	1.18	1.13	1.10	1.11	1.09	1.04
119.0	1.31	1.22	1.21	1.18	1.13	1.10	1.10	1.09	1.04
119.5	1.30	1.23	1.21	1.18	1.14	1.10	1.11	1.09	1.05
120.0	1.31	1.23	1.21	1.18	1.13	1.10	1.11	1.09	1.04
120.5	1.30	1.23	1.20	1.18	1.13	1.10	1.10	1.09	1.04
121.0	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.09	1.04
121.5	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.09	1.04
122.0	1.30	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04
122.5	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04
123.0	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
123.5	1.31	1.24	1.21	1.18	1.13	1.10	1.10	1.08	1.04
124.0	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04
124.5	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.09	1.04
125.0	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04
125.5	1.32	1.24	1.21	1.18	1.13	1.10	1.10	1.09	1.04
126.0	1.31	1.24	1.21	1.18	1.13	1.10	1.10	1.08	1.04
126.5	1.31	1.23	1.21	1.18	1.13	1.10	1.10	1.08	1.04
127.0	1.32	1.24	1.21	1.19	1.13	1.11	1.11	1.09	1.04
127.5	1.32	1.24	1.21	1.19	1.13	1.10	1.11	1.09	1.04
128.0	1.32	1.24	1.21	1.18	1.13	1.10	1.11	1.09	1.04
128.5	1.32	1.24	1.21	1.18	1.13	1.10	1.10	1.08	1.04
129.0	1.32	1.24	1.21	1.18	1.13	1.10	1.11	1.09	1.04
129.5	1.32	1.24	1.22	1.18	1.13	1.10	1.10	1.09	1.04
130.0	1.32	1.24	1.21	1.19	1.13	1.10	1.10	1.09	1.04
130.5	1.32	1.24	1.21	1.18	1.13	1.10	1.10	1.08	1.04
131.0	1.32	1.24	1.21	1.19	1.13	1.10	1.10	1.08	1.04
131.5	1.32	1.24	1.21	1.19	1.13	1.10	1.10	1.09	1.04
132.0	1.32	1.24	1.22	1.18	1.13	1.10	1.10	1.08	1.04
132.5	1.32	1.24	1.21	1.18	1.13	1.10	1.10	1.08	1.04
133.0	1.32	1.24	1.22	1.18	1.13	1.10	1.10	1.08	1.04
133.5	1.32	1.24	1.22	1.18	1.13	1.10	1.11	1.08	1.04
134.0	1.32	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
134.5	1.33	1.24	1.22	1.18	1.13	1.10	1.10	1.08	1.04
135.0	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
135.5	1.32	1.24	1.22	1.19	1.13	1.10	1.10	1.08	1.04
136.0	1.33	1.25	1.22	1.19	1.14	1.10	1.10	1.09	1.04
136.5	1.33	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
137.0	1.32	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
137.5	1.32	1.24	1.22	1.19	1.13	1.10	1.10	1.08	1.04
138.0	1.33	1.25	1.22	1.19	1.14	1.11	1.11	1.09	1.04
138.5	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
139.0	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
139.5	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
140.0	1.34	1.25	1.23	1.19	1.14	1.10	1.11	1.08	1.04
140.5	1.33	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
141.0	1.33	1.25	1.22	1.19	1.14	1.10	1.10	1.08	1.04
141.5	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
142.0	1.33	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
142.5	1.33	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
143.0	1.33	1.25	1.22	1.19	1.13	1.10	1.11	1.08	1.04
143.5	1.33	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
144.0	1.33	1.25	1.23	1.19	1.13	1.10	1.10	1.08	1.04
144.5	1.34	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
145.0	1.34	1.25	1.23	1.19	1.13	1.10	1.11	1.08	1.04
145.5	1.33	1.25	1.23	1.19	1.13	1.10	1.11	1.08	1.04
146.0	1.34	1.25	1.22	1.19	1.13	1.10	1.10	1.08	1.04
146.5	1.34	1.26	1.23	1.20	1.13	1.10	1.10	1.08	1.04
147.0	1.34	1.25	1.23	1.19	1.13	1.10	1.11	1.08	1.04
147.5	1.34	1.25	1.23	1.19	1.14	1.10	1.10	1.08	1.04
148.0	1.34	1.26	1.23	1.20	1.13	1.10	1.10	1.08	1.04
148.5	1.34	1.26	1.23	1.19	1.14	1.10	1.10	1.08	1.04
149.0	1.33	1.26	1.23	1.19	1.14	1.10	1.10	1.08	1.04
149.5	1.34	1.26	1.23	1.19	1.13	1.10	1.10	1.08	1.04

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
150.0	1.34	1.26	1.22	1.19	1.13	1.10	1.10	1.08	1.04
150.5	1.34	1.26	1.23	1.19	1.14	1.10	1.11	1.08	1.04
151.0	1.34	1.26	1.23	1.19	1.14	1.10	1.11	1.08	1.04
151.5	1.34	1.26	1.23	1.19	1.13	1.10	1.10	1.08	1.04
152.0	1.34	1.25	1.23	1.19	1.14	1.10	1.10	1.08	1.04
152.5	1.34	1.26	1.23	1.19	1.14	1.10	1.10	1.08	1.04
153.0	1.34	1.26	1.23	1.19	1.14	1.10	1.10	1.08	1.04
153.5	1.34	1.26	1.23	1.19	1.13	1.10	1.10	1.08	1.04
154.0	1.34	1.26	1.23	1.19	1.13	1.10	1.10	1.08	1.04
154.5	1.34	1.26	1.23	1.19	1.14	1.10	1.11	1.08	1.04
155.0	1.34	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
155.5	1.35	1.26	1.23	1.20	1.13	1.11	1.11	1.08	1.04
156.0	1.34	1.26	1.23	1.19	1.13	1.10	1.10	1.08	1.04
156.5	1.35	1.26	1.23	1.20	1.13	1.10	1.10	1.08	1.04
157.0	1.35	1.26	1.23	1.19	1.14	1.10	1.10	1.08	1.04
157.5	1.35	1.27	1.24	1.20	1.14	1.10	1.11	1.08	1.04
158.0	1.35	1.26	1.23	1.20	1.13	1.10	1.10	1.08	1.04
158.5	1.35	1.26	1.23	1.20	1.14	1.10	1.10	1.08	1.04
159.0	1.34	1.27	1.23	1.19	1.13	1.10	1.10	1.08	1.03
159.5	1.35	1.26	1.23	1.20	1.13	1.10	1.10	1.08	1.04
160.0	1.34	1.26	1.23	1.20	1.14	1.10	1.10	1.08	1.04
160.5	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
161.0	1.35	1.26	1.24	1.20	1.14	1.10	1.11	1.08	1.04
161.5	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
162.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
162.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
163.0	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
163.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
164.0	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
164.5	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.03
165.0	1.35	1.27	1.24	1.20	1.13	1.10	1.10	1.08	1.04
165.5	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.03
166.0	1.35	1.27	1.24	1.20	1.13	1.10	1.10	1.08	1.04
166.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
167.0	1.35	1.27	1.24	1.20	1.13	1.10	1.10	1.08	1.03
167.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
168.0	1.35	1.27	1.24	1.20	1.13	1.10	1.10	1.08	1.04
168.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
169.0	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
169.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.04
170.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
170.5	1.35	1.26	1.24	1.20	1.14	1.10	1.10	1.08	1.04
171.0	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.03
171.5	1.35	1.27	1.24	1.20	1.13	1.10	1.10	1.07	1.03
172.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
172.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.03
173.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
173.5	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
174.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
174.5	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
175.0	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.03
175.5	1.35	1.27	1.25	1.20	1.14	1.10	1.10	1.07	1.03
176.0	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.03

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
176.5	1.36	1.28	1.24	1.21	1.14	1.10	1.10	1.08	1.04
177.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.03
177.5	1.36	1.27	1.25	1.20	1.14	1.10	1.10	1.07	1.04
178.0	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.03
178.5	1.35	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.03
179.0	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.04
179.5	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.08	1.04
180.0	1.36	1.27	1.25	1.21	1.14	1.10	1.10	1.07	1.03
180.5	1.36	1.28	1.24	1.20	1.14	1.10	1.10	1.07	1.04
181.0	1.36	1.27	1.25	1.20	1.14	1.10	1.10	1.08	1.04
181.5	1.36	1.27	1.25	1.20	1.14	1.10	1.10	1.07	1.03
182.0	1.36	1.27	1.25	1.21	1.14	1.10	1.10	1.07	1.04
182.5	1.36	1.27	1.25	1.21	1.14	1.10	1.10	1.07	1.04
183.0	1.36	1.28	1.24	1.20	1.14	1.10	1.10	1.07	1.04
183.5	1.37	1.28	1.25	1.20	1.14	1.10	1.10	1.08	1.03
184.0	1.36	1.27	1.24	1.20	1.13	1.10	1.10	1.07	1.03
184.5	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
185.0	1.36	1.28	1.25	1.20	1.14	1.09	1.10	1.07	1.04
185.5	1.36	1.27	1.25	1.21	1.14	1.10	1.10	1.07	1.03
186.0	1.36	1.27	1.25	1.20	1.14	1.10	1.10	1.08	1.04
186.5	1.36	1.27	1.25	1.20	1.14	1.09	1.10	1.07	1.03
187.0	1.36	1.28	1.24	1.20	1.14	1.10	1.10	1.07	1.03
187.5	1.36	1.27	1.24	1.20	1.14	1.10	1.10	1.07	1.03
188.0	1.36	1.28	1.25	1.20	1.14	1.09	1.10	1.07	1.03
188.5	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
189.0	1.36	1.27	1.25	1.20	1.14	1.10	1.10	1.07	1.03
189.5	1.36	1.27	1.25	1.20	1.13	1.10	1.09	1.07	1.03
190.0	1.37	1.28	1.25	1.20	1.14	1.10	1.10	1.07	1.04
190.5	1.37	1.28	1.25	1.21	1.14	1.10	1.10	1.08	1.04
191.0	1.36	1.28	1.25	1.20	1.14	1.10	1.10	1.07	1.03
191.5	1.36	1.27	1.25	1.20	1.13	1.10	1.09	1.07	1.03
192.0	1.36	1.27	1.25	1.21	1.14	1.10	1.10	1.07	1.03
192.5	1.37	1.28	1.25	1.20	1.14	1.10	1.10	1.07	1.03
193.0	1.37	1.28	1.25	1.21	1.14	1.10	1.10	1.08	1.03
193.5	1.36	1.27	1.25	1.20	1.14	1.10	1.09	1.07	1.03
194.0	1.37	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.04
194.5	1.36	1.27	1.25	1.20	1.13	1.09	1.09	1.07	1.03
195.0	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
195.5	1.36	1.28	1.25	1.20	1.14	1.10	1.09	1.07	1.03
196.0	1.37	1.28	1.26	1.21	1.14	1.10	1.10	1.07	1.04
196.5	1.36	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
197.0	1.37	1.28	1.26	1.21	1.14	1.10	1.10	1.07	1.03
197.5	1.37	1.28	1.25	1.21	1.13	1.09	1.09	1.07	1.03
198.0	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
198.5	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
199.0	1.36	1.27	1.25	1.21	1.14	1.10	1.09	1.07	1.03
199.5	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
200.0	1.37	1.28	1.25	1.21	1.14	1.09	1.10	1.07	1.03
200.5	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
201.0	1.37	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
201.5	1.36	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
202.0	1.37	1.28	1.26	1.21	1.14	1.10	1.10	1.07	1.04
202.5	1.37	1.28	1.25	1.20	1.14	1.10	1.09	1.07	1.03

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
203.0	1.37	1.28	1.25	1.21	1.14	1.10	1.10	1.07	1.03
203.5	1.37	1.28	1.25	1.21	1.13	1.09	1.09	1.07	1.03
204.0	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
204.5	1.37	1.28	1.26	1.21	1.14	1.10	1.10	1.07	1.03
205.0	1.36	1.28	1.25	1.21	1.13	1.09	1.09	1.07	1.03
205.5	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.07	1.04
206.0	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
206.5	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
207.0	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
207.5	1.36	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
208.0	1.37	1.28	1.26	1.21	1.14	1.10	1.10	1.07	1.03
208.5	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.07	1.03
209.0	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
209.5	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
210.0	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
210.5	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.06	1.03
211.0	1.37	1.28	1.25	1.21	1.14	1.10	1.09	1.06	1.03
211.5	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
212.0	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.06	1.03
212.5	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
213.0	1.37	1.29	1.25	1.21	1.14	1.10	1.09	1.07	1.03
213.5	1.37	1.29	1.26	1.21	1.14	1.09	1.10	1.07	1.03
214.0	1.37	1.29	1.26	1.21	1.14	1.10	1.09	1.07	1.03
214.5	1.37	1.29	1.25	1.21	1.14	1.09	1.09	1.07	1.03
215.0	1.37	1.28	1.25	1.21	1.13	1.09	1.09	1.07	1.03
215.5	1.37	1.29	1.26	1.21	1.14	1.09	1.09	1.07	1.03
216.0	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
216.5	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
217.0	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
217.5	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.07	1.03
218.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.07	1.03
218.5	1.38	1.29	1.26	1.21	1.14	1.10	1.09	1.07	1.03
219.0	1.37	1.28	1.25	1.21	1.14	1.09	1.09	1.07	1.03
219.5	1.38	1.28	1.26	1.21	1.14	1.10	1.09	1.07	1.03
220.0	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
220.5	1.38	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
221.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
221.5	1.37	1.29	1.26	1.21	1.14	1.10	1.09	1.07	1.03
222.0	1.38	1.29	1.26	1.21	1.14	1.09	1.10	1.07	1.03
222.5	1.37	1.29	1.26	1.21	1.14	1.10	1.09	1.07	1.03
223.0	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
223.5	1.37	1.28	1.25	1.21	1.13	1.09	1.09	1.06	1.02
224.0	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.07	1.02
224.5	1.38	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
225.0	1.38	1.28	1.26	1.21	1.14	1.09	1.09	1.07	1.03
225.5	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
226.0	1.38	1.28	1.26	1.21	1.14	1.10	1.09	1.06	1.03
226.5	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
227.0	1.38	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
227.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
228.0	1.37	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.02
228.5	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
229.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.07	1.03

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
229.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.07	1.03
230.0	1.37	1.28	1.26	1.21	1.14	1.09	1.09	1.06	1.03
230.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
231.0	1.37	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.02
231.5	1.38	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
232.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
232.5	1.37	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
233.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
233.5	1.37	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
234.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
234.5	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.06	1.03
235.0	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.06	1.03
235.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
236.0	1.38	1.29	1.26	1.21	1.14	1.09	1.08	1.06	1.03
236.5	1.37	1.28	1.26	1.21	1.14	1.10	1.09	1.06	1.03
237.0	1.37	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
237.5	1.38	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
238.0	1.38	1.29	1.27	1.21	1.14	1.09	1.09	1.06	1.03
238.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
239.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
239.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
240.0	1.37	1.28	1.26	1.21	1.14	1.09	1.08	1.06	1.03
240.5	1.37	1.28	1.26	1.21	1.13	1.09	1.09	1.06	1.03
241.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
241.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.06	1.03
242.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
242.5	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.06	1.03
243.0	1.37	1.28	1.26	1.21	1.13	1.09	1.08	1.06	1.03
243.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.02
244.0	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
244.5	1.38	1.29	1.26	1.21	1.14	1.09	1.09	1.06	1.03
245.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.06	1.03
245.5	1.38	1.29	1.27	1.21	1.14	1.09	1.09	1.06	1.02
246.0	1.38	1.29	1.26	1.21	1.14	1.09	1.08	1.06	1.03
246.5	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.06	1.03
247.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.06	1.02
247.5	1.38	1.28	1.26	1.21	1.13	1.09	1.08	1.05	1.02
248.0	1.38	1.29	1.27	1.21	1.13	1.09	1.09	1.06	1.03
248.5	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.06	1.02
249.0	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.05	1.03
249.5	1.37	1.29	1.27	1.21	1.14	1.09	1.09	1.05	1.02
250.0	1.37	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
250.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
251.0	1.38	1.29	1.27	1.21	1.14	1.09	1.09	1.05	1.03
251.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
252.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
252.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.03
253.0	1.38	1.29	1.27	1.21	1.13	1.09	1.09	1.06	1.03
253.5	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.03
254.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.02
254.5	1.38	1.29	1.26	1.21	1.14	1.09	1.08	1.05	1.03
255.0	1.39	1.29	1.27	1.21	1.14	1.09	1.09	1.05	1.03
255.5	1.38	1.29	1.27	1.21	1.14	1.09	1.08	1.06	1.03

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
256.0	1.39	1.29	1.26	1.21	1.14	1.09	1.08	1.05	1.02
256.5	1.38	1.29	1.26	1.21	1.13	1.09	1.09	1.05	1.03
257.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
257.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.03
258.0	1.38	1.28	1.27	1.21	1.13	1.08	1.08	1.05	1.02
258.5	1.38	1.29	1.26	1.21	1.14	1.09	1.08	1.05	1.02
259.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
259.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
260.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.03
260.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
261.0	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.05	1.03
261.5	1.37	1.28	1.26	1.21	1.13	1.09	1.08	1.05	1.02
262.0	1.38	1.29	1.26	1.21	1.14	1.09	1.08	1.05	1.03
262.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
263.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.03
263.5	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.03
264.0	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
264.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
265.0	1.37	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
265.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
266.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
266.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
267.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.02
267.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
268.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
268.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.03
269.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
269.5	1.38	1.29	1.26	1.21	1.13	1.09	1.08	1.05	1.02
270.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.02
270.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
271.0	1.38	1.29	1.27	1.21	1.12	1.08	1.08	1.05	1.02
271.5	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.05	1.02
272.0	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.05	1.02
272.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.05	1.02
273.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.02
273.5	1.38	1.29	1.26	1.21	1.12	1.08	1.08	1.05	1.02
274.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.05	1.03
274.5	1.38	1.29	1.26	1.21	1.13	1.09	1.07	1.05	1.02
275.0	1.38	1.29	1.27	1.21	1.13	1.09	1.08	1.04	1.02
275.5	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.04	1.02
276.0	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.04	1.02
276.5	1.37	1.29	1.26	1.20	1.13	1.08	1.07	1.04	1.02
277.0	1.38	1.29	1.27	1.21	1.13	1.09	1.07	1.04	1.02
277.5	1.38	1.28	1.26	1.21	1.13	1.08	1.07	1.04	1.02
278.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
278.5	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.04	1.02
279.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
279.5	1.38	1.28	1.27	1.21	1.12	1.08	1.08	1.04	1.02
280.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
280.5	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
281.0	1.38	1.29	1.26	1.20	1.13	1.08	1.08	1.04	1.02
281.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
282.0	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
282.5	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
283.0	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
283.5	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.04	1.02
284.0	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.04	1.02
284.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
285.0	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.05	1.02
285.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
286.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
286.5	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
287.0	1.38	1.29	1.26	1.21	1.13	1.08	1.08	1.04	1.02
287.5	1.38	1.29	1.27	1.20	1.13	1.08	1.07	1.04	1.02
288.0	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
288.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
289.0	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
289.5	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
290.0	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
290.5	1.37	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
291.0	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.04	1.02
291.5	1.38	1.29	1.27	1.21	1.13	1.08	1.08	1.04	1.02
292.0	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
292.5	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.04	1.02
293.0	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
293.5	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
294.0	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.04	1.02
294.5	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
295.0	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
295.5	1.38	1.29	1.27	1.20	1.13	1.08	1.07	1.04	1.02
296.0	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.03	1.02
296.5	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
297.0	1.38	1.29	1.27	1.21	1.13	1.08	1.07	1.04	1.02
297.5	1.38	1.29	1.26	1.21	1.13	1.08	1.07	1.04	1.02
298.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
298.5	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.02
299.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
299.5	1.37	1.29	1.26	1.21	1.12	1.08	1.07	1.04	1.02
300.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
300.5	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
301.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
301.5	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
302.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
302.5	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.04	1.01
303.0	1.37	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
303.5	1.37	1.29	1.26	1.21	1.12	1.08	1.07	1.04	1.02
304.0	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.04	1.02
304.5	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
305.0	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
305.5	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.04	1.02
306.0	1.37	1.28	1.26	1.20	1.12	1.07	1.06	1.03	1.01
306.5	1.37	1.29	1.26	1.20	1.12	1.07	1.07	1.03	1.02
307.0	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
307.5	1.37	1.29	1.27	1.20	1.12	1.07	1.07	1.04	1.02
308.0	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
308.5	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.04	1.02

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
309.0	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.04	1.02
309.5	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.04	1.02
310.0	1.37	1.29	1.26	1.21	1.12	1.07	1.06	1.03	1.02
310.5	1.38	1.29	1.26	1.21	1.11	1.08	1.07	1.03	1.02
311.0	1.37	1.29	1.26	1.20	1.12	1.07	1.07	1.03	1.02
311.5	1.38	1.29	1.26	1.21	1.12	1.07	1.07	1.04	1.02
312.0	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
312.5	1.37	1.28	1.26	1.20	1.12	1.08	1.07	1.03	1.02
313.0	1.38	1.29	1.27	1.20	1.12	1.08	1.07	1.03	1.01
313.5	1.37	1.28	1.26	1.20	1.12	1.08	1.07	1.03	1.02
314.0	1.38	1.29	1.27	1.21	1.12	1.08	1.07	1.03	1.02
314.5	1.38	1.29	1.26	1.20	1.12	1.07	1.07	1.03	1.02
315.0	1.37	1.29	1.27	1.20	1.12	1.08	1.07	1.03	1.02
315.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
316.0	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.03	1.02
316.5	1.38	1.28	1.26	1.20	1.12	1.08	1.06	1.03	1.01
317.0	1.38	1.29	1.26	1.21	1.12	1.07	1.07	1.04	1.02
317.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
318.0	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
318.5	1.37	1.29	1.26	1.20	1.12	1.07	1.07	1.03	1.02
319.0	1.38	1.29	1.26	1.21	1.12	1.08	1.07	1.03	1.02
319.5	1.38	1.29	1.27	1.20	1.12	1.07	1.07	1.03	1.01
320.0	1.37	1.29	1.27	1.20	1.12	1.07	1.07	1.03	1.02
320.5	1.37	1.28	1.26	1.20	1.12	1.08	1.07	1.03	1.02
321.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
321.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
322.0	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
322.5	1.37	1.29	1.26	1.20	1.12	1.08	1.06	1.03	1.02
323.0	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
323.5	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
324.0	1.38	1.28	1.27	1.20	1.12	1.07	1.06	1.03	1.02
324.5	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.03	1.01
325.0	1.38	1.28	1.26	1.20	1.12	1.07	1.07	1.03	1.02
325.5	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
326.0	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
326.5	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.03	1.02
327.0	1.38	1.29	1.26	1.21	1.12	1.07	1.06	1.03	1.02
327.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
328.0	1.38	1.29	1.26	1.20	1.12	1.08	1.06	1.03	1.01
328.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
329.0	1.38	1.29	1.26	1.21	1.12	1.08	1.06	1.03	1.02
329.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
330.0	1.38	1.29	1.27	1.20	1.12	1.08	1.06	1.03	1.02
330.5	1.38	1.29	1.27	1.20	1.12	1.08	1.06	1.03	1.02
331.0	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
331.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
332.0	1.37	1.29	1.27	1.20	1.12	1.08	1.06	1.03	1.02
332.5	1.37	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
333.0	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
333.5	1.38	1.28	1.26	1.20	1.11	1.07	1.06	1.03	1.01
334.0	1.38	1.29	1.26	1.20	1.12	1.08	1.06	1.03	1.02
334.5	1.38	1.29	1.26	1.20	1.12	1.08	1.07	1.03	1.02
335.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
335.5	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
336.0	1.38	1.29	1.27	1.20	1.12	1.08	1.06	1.03	1.01
336.5	1.38	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
337.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
337.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.03	1.01
338.0	1.37	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
338.5	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.01
339.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
339.5	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
340.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.02	1.01
340.5	1.37	1.28	1.26	1.20	1.12	1.07	1.06	1.03	1.01
341.0	1.38	1.28	1.26	1.20	1.12	1.07	1.06	1.03	1.01
341.5	1.37	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.01
342.0	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
342.5	1.37	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
343.0	1.37	1.28	1.26	1.20	1.12	1.07	1.06	1.03	1.02
343.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
344.0	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.01
344.5	1.37	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.02
345.0	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
345.5	1.37	1.29	1.26	1.20	1.12	1.07	1.06	1.03	1.02
346.0	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.02
346.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.02
347.0	1.37	1.29	1.27	1.20	1.11	1.07	1.06	1.03	1.02
347.5	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
348.0	1.37	1.29	1.27	1.20	1.11	1.07	1.06	1.02	1.01
348.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.03	1.01
349.0	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.03	1.01
349.5	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
350.0	1.38	1.29	1.27	1.20	1.12	1.07	1.06	1.03	1.01
350.5	1.38	1.30	1.27	1.20	1.12	1.07	1.06	1.03	1.02
351.0	1.37	1.29	1.27	1.20	1.11	1.07	1.06	1.02	1.01
351.5	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
352.0	1.38	1.29	1.27	1.20	1.11	1.07	1.06	1.02	1.02
352.5	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
353.0	1.38	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
353.5	1.38	1.29	1.27	1.20	1.11	1.07	1.06	1.03	1.02
354.0	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.01
354.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.02
355.0	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
355.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
356.0	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.01
356.5	1.38	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
357.0	1.37	1.28	1.27	1.20	1.11	1.07	1.06	1.02	1.02
357.5	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
358.0	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
358.5	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.02
359.0	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
359.5	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
360.0	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
360.5	1.37	1.29	1.26	1.20	1.11	1.06	1.06	1.02	1.01
361.0	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
361.5	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
362.0	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
362.5	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.02
363.0	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.01
363.5	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
364.0	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
364.5	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
365.0	1.37	1.29	1.27	1.20	1.11	1.06	1.05	1.02	1.01
365.5	1.38	1.29	1.27	1.20	1.11	1.07	1.05	1.02	1.01
366.0	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
366.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
367.0	1.37	1.28	1.26	1.20	1.11	1.07	1.06	1.02	1.01
367.5	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.01
368.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
368.5	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.02
369.0	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
369.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
370.0	1.37	1.28	1.26	1.20	1.11	1.07	1.05	1.02	1.01
370.5	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
371.0	1.37	1.29	1.26	1.20	1.11	1.07	1.06	1.02	1.01
371.5	1.38	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
372.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.02
372.5	1.37	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
373.0	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
373.5	1.37	1.28	1.26	1.19	1.11	1.07	1.05	1.02	1.01
374.0	1.37	1.29	1.26	1.19	1.11	1.06	1.06	1.02	1.01
374.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
375.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
375.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
376.0	1.36	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
376.5	1.37	1.28	1.26	1.20	1.10	1.06	1.05	1.02	1.01
377.0	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
377.5	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
378.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
378.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
379.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
379.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
380.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.02
380.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
381.0	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.01	1.01
381.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
382.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
382.5	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
383.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
383.5	1.36	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
384.0	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
384.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
385.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
385.5	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.02	1.01
386.0	1.37	1.28	1.26	1.20	1.10	1.06	1.04	1.01	1.01
386.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
387.0	1.36	1.28	1.26	1.19	1.11	1.06	1.05	1.01	1.01
387.5	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
388.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01

TABLE 9
Ligand Concentration Dependency of NR1/2B-Mediated Calcium Flux

<u>time</u> <u>sec</u>	<u>100.00</u> <u>μM</u>	<u>50.00</u> <u>μM</u>	<u>25.00</u> <u>μM</u>	<u>12.50</u> <u>μM</u>	<u>6.25</u> <u>μM</u>	<u>3.13</u> <u>μM</u>	<u>1.67</u> <u>μM</u>	<u>0.84</u> <u>μM</u>	<u>no</u> <u>ligand</u>
388.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.01	1.01
389.0	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
389.5	1.36	1.28	1.26	1.19	1.10	1.06	1.04	1.01	1.01
390.0	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
390.5	1.36	1.28	1.26	1.19	1.10	1.06	1.05	1.01	1.01
391.0	1.37	1.29	1.26	1.19	1.11	1.06	1.05	1.02	1.01
391.5	1.36	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
392.0	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.02	1.01
392.5	1.37	1.28	1.26	1.19	1.11	1.06	1.05	1.01	1.01
393.0	1.36	1.29	1.26	1.20	1.11	1.07	1.05	1.02	1.01
393.5	1.36	1.28	1.26	1.19	1.11	1.06	1.04	1.01	1.01
394.0	1.37	1.29	1.26	1.19	1.11	1.06	1.05	1.02	1.01
394.5	1.37	1.29	1.26	1.19	1.11	1.06	1.05	1.01	1.01
395.0	1.37	1.29	1.26	1.20	1.11	1.06	1.05	1.01	1.01
395.5	1.36	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
396.0	1.37	1.28	1.26	1.20	1.11	1.06	1.05	1.02	1.01
396.5	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
397.0	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.01	1.01
397.5	1.36	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.01
398.0	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.02	1.02
398.5	1.36	1.28	1.26	1.19	1.11	1.06	1.05	1.01	1.01
399.0	1.37	1.28	1.26	1.19	1.10	1.06	1.05	1.01	1.01

Concentrations and data rounded to two decimal places

[0101] NR1/2B showed overall weaker signal and slower kinetics as compared to NR1/2A, as has previously observed in electrophysiological assays. NR1/2B activity was dependent on the presence of both subunits and optimal functional expression levels were obtained at a MOI of 250xNR1:1000xNR2A and was used for all subsequent experiments.

[0102] TABLE 10 shows the titration of the maximal NMDAR-mediated calcium flux in HEK293 cells transduced with baculovirus. Cells were transduced with different ratios/amounts of NR1 and NR2B and NMDAR-activity was assessed as in TABLE 6.

TABLE 10 Titration of the Maximal NMDAR-Mediated Calcium Flux in HEK293 Cells Transduced with Baculovirus						
<u>NR1</u> <u>(MOI)</u>	<u>NR2B (MOI)</u>					
	<u>0</u>	<u>0</u>	<u>2000</u>	<u>2000</u>	<u>500</u>	<u>250</u>
	<u>0</u>	1.0	1.0	1.0	1.0	1.0
	<u>1000</u>	1.0	1.7	1.8	1.9	1.8
	<u>500</u>	1.0	1.8	1.9	2.0	1.9
	<u>250</u>	1.0	1.9	2.1	2.0	1.9
	<u>125</u>	1.0	1.9	1.9	1.8	1.8

[0103] Taken together, these data shows that the assay conditions that we identified for NR1/2A and NR1/2B can now be transferred to other NMDAR subunits. This shows the

flexibility and advantage of baculovirus mediated expression of NMDAR and establishes the assay of the invention to study NR1/2B activity in non-neuronal cells.

EXAMPLE IV

- 5 *An unbiased screen identifies MDL105,519 and CGP070667 to protect from excitotoxicity and to facilitate the measurement of NR1/2A- mediated and NR1/2B-mediated calcium flux*

[0104] We had found that MDL105,519 was able to protect the cells from excitotoxicity and facilitated the measurement of NMDAR activity. We then performed a screen using
10 ~130 known inhibitors of the NMDAR receptor complex and their corresponding structural analogues. The aim of the screen was to identify compounds that (1) could increase cell viability; (2) could be readily removed upon washing; and (3) enabled subsequent detection of NMDAR mediated calcium flux in our FDSS assay. HEK293 cells were transduced with NR1/2A or NR1/2B and treated with compound at four
15 different concentrations (100 μ M, 33 μ M, 11 μ M and 3 μ M). Sixteen hours after transduction, the cells were washed twice in assay buffer and NMDAR activity was measured in the FDSS using calcium dye and stimulation with saturating amount of glycine and glutamate. Cell viability was assessed after the FDSS measurement using CellTiter-Glo®.

20 **[0105]** Besides MDL105,519, only CHEMBL276708 or (2,3-dioxo-1,4-dihydroquinoxalin-5-yl)methylphosphonic acid (see, CHEMBL276708 at the PubChem OPEN CHEMISTRY DATABASE, CID 44268212), 5-phosphonomethyl-1,4-dihydroquinoxaline-2,3-dione (Auberson YP *et al.*, *Bioorg. Med. Chem. Lett.* 12:1099-102 (2002)), and the glutamate binding site antagonist CGP070667 (Auberson YP *et al.*,
25 *Bioorg. Med. Chem. Lett.* 12:1099-102 (2002)), as well as its structural analogs, protected the cells from excitotoxicity and retained the ability to measure both NR1/2A- and NR1/2B-mediated calcium flux. MDL105,519 (100 μ M), MK801 (10 μ M) and ketamine (1 mM) were used as controls. While treatment with ketamine and MK-801 protected the cells from excitotoxicity, subsequent calcium measurement after washing was not
30 possible.

[0106] Taken together, our data demonstrates that baculovirus mediated expression of NMDAR in combination with the treatment with the ligand binding-site antagonists MDL105,519 and CHEMBL276708, can be as a system to study NR1/2A-activity and NR1/2B-activity.

EXAMPLE VBaculovirus mediated expression of NMDAR in combination with antagonist protection can be used to study NMDAR-biology and pharmacology

5 [0107] Having established the ability to reduce cell loss due to NMDAR cytotoxicity and to retain the ability to measure Ca^{2+} signaling, we determined that the system of the invention faithfully replicated expected NMDAR biology and pharmacology. To this end, we studied the effects to membrane potential on NMDAR activity as well as profiled several known NMDAR antagonists and allosteric modulators.

10 [0108] One hallmark of NMDAR is the membrane potential dependent blockade of channel activity by magnesium. In neuronal cells, NMDAR activity is almost completely blocked by Mg^{2+} , due to the negative resting membrane potential of neuronal cells (~ -70 mV). Depolarization of the membrane results in release of Mg^{2+} and channel activity, thus establishing the voltage-gated regulation of NMDAR. HEK293 cells are known to have a depolarized membrane resting potential as compared to neuronal cells. We
15 determined a resting membrane potential of ~ -30 mV in the HEK293 cells, so Mg^{2+} is not expected to contribute to NMDAR regulation under these conditions.

[0109] However, hyperpolarization of the membrane potential in these cells is expected to result in an increased sensitivity to inhibition by Mg^{2+} . As opposed to electrophysiological techniques, a direct control of the membrane potential of the cells in
20 FDSS measurements is not possible. To modify the resting membrane potential of the cells and to study the effects of Mg^{2+} in our system, we expressed different amounts of the KCNJ2 (Kir2.1) receptor, an integral membrane receptor that allows inward flow of K^{+} into the cell, via baculovirus in addition to the NR1/2A. Increasing expression of KCNJ2 is expected to result in a hyperpolarization of the cells' resting potential.

[0110] TABLE 8 shows that increasing levels of KCNJ2 in the cells resulted in increased sensitivity of NR1/2A activity to Mg^{2+} , consistent with the expected behavior of these receptors in neurons. We transduced HEK293 cells with baculovirus vector encoding for KCNJ2 (Kir2.1), NR1 and NR2A in the presence of MDL105,519 and NMDAR-mediated calcium flux was measured as described above in the presence of varying amounts of magnesium chloride. The maximal fluorescence ratio was normalized to NR1/2A-transduced cells in the absence of magnesium. Data in TABLE 11 represent the mean $\pm$ STDEV of a representative experiment.

TABLE 11
Sensitivity of NMDAR-Mediated Calcium Flux to Inhibition
by Magnesium at Varying Membrane Potentials

concentration	0 μ l Kir2.1				1 μ l Kir2.1			
MgCl ₂ (mM)								
33.00	0.45	0.44	0.46	0.42	0.42	0.43	0.42	0.39
11.00	0.48	0.45	0.51	0.46	0.43	0.37	0.40	0.39
3.30	0.61	0.60	0.59	0.57	0.43	0.45	0.45	0.43
1.10	0.61	0.72	0.73	0.73	0.50	0.49	0.53	0.48
0.33	0.97	0.86	0.96	0.83	0.62	0.61	0.67	0.58
0.11	0.90	0.93	1.03	0.99	0.80	0.83	0.91	0.90
0.03	1.13	0.99	0.91	0.96	1.04	0.97	0.93	1.12
0.01	0.90	0.93	1.07	1.03	1.03	1.01	1.18	1.09
		2 μ l Kir2.1			4 μ l Kir2.1			
33.00	0.41	0.40	0.41	0.39	0.41	0.40	0.38	0.37
11.00	0.37	0.37	0.37	0.39	0.36	0.36	0.38	0.37
3.30	0.40	0.42	0.40	0.43	0.40	0.38	0.41	0.38
1.10	0.50	0.44	0.45	0.51	0.41	0.44	0.43	0.44
0.33	0.51	0.53	0.51	0.59	0.47	0.46	0.47	0.49
0.11	0.69	0.67	0.68	0.72	0.55	0.62	0.57	0.61
0.03	0.80	0.79	0.76	0.85	0.68	0.70	0.63	0.76
0.01	0.78	0.99	0.92	0.97	0.71	0.73	0.73	0.77
		6 μ l Kir2.1			8 μ l Kir2.1			
33.00	0.41	0.41	0.38	0.39	0.40	0.40	0.38	0.40
11.00	0.36	0.34	0.36	0.35	0.35	0.37	0.35	0.36
3.30	0.38	0.36	0.39	0.39	0.35	0.39	0.38	0.40
1.10	0.39	0.40	0.41	0.39	0.36	0.41	0.39	0.40
0.33	0.44	0.42	0.44	0.43	0.38	0.41	0.40	0.43
0.11	0.48	0.49	0.47	0.49	0.41	0.46	0.47	0.48
0.03	0.56	0.55	0.58	0.52	0.49	0.50	0.52	0.54
0.01	0.62	0.65	0.69	0.73	0.58	0.58	0.70	0.66

Data was rounded to two decimal places

10 **[0111]** These data demonstrate an easy and rapid method and assay to study the membrane potential-dependent effect of magnesium on NMDAR activity in non-neuronal cells other than electrophysiology.

[0112] Next, we tested whether known pharmacology could be reproduced with compounds that target the NMDAR complex, in particular NR1/2A and NR1/2B. HEK293 cells were transduced with NR1/2A or NR1/2B, protected with MDL105,159 and NMDAR activity in the presence of varying concentrations of NMDAR inhibitors was measured in the FDSS assay. For this, cells were preincubated with the compound for five minutes. NMDAR activity was determined after ligand addition using the area under the curve (fluorescence ratio) as readout.

[0113] TABLE 12 lists some compounds tested, their mechanisms of action and the IC₅₀ values obtained in our system. The data in TABLE 12 represent the mean of the IC₅₀ (μM) as determined in NR1/2A transduced HEK293 cells after protection with MDL105.519 in n>3 experiments after stimulation with EC₈₀ of both ligands.

TABLE 12				
Measured IC ₅₀ values of NMDAR inhibitors				
Compound	Mode of Action	Reference	NR2A	NR2B
TCN201	negative allosteric modulator, NR2A selective		11.93	n.d.
CGP070667	Glutamate site antagonist, weakly NR2A selective		0.07	0.37
AAM077	Glutamate site antagonist, weakly NR2A selective		2.46	11.86
CP101606	NR2B antagonist		n.d.	0.02
RO25-6981	NR2B antagonist		n.d.	0.10
Ifenprodil	negative allosteric modulator, NR2B selective		n.d.	0.10
7-CTKA	glycine site antagonist		28.02	28.32
MDL105,519	glycine site antagonist		0.62	1.65
L701,324	glycine site antagonist		1.45	1.91
CGP039653	glutamate site antagonist		9.59	38.90
CPP	glutamate site antagonist		n.d.	n.d.
MK801	channel blocker		0.10	0.15
ketamine	channel blocker		23.00	12.14
QNZ46	NR2C/2D selective antagonist		n.d.	n.d.

Data were rounded to two decimal places

[0114] Taken together, our data shows that the assay and methods of the invention reproduced the expected NMDAR pharmacology. This shows that the assay is both biologically and pharmacologically relevant.

[0115] In summary, protection of NMDAR-expressing cells from excitotoxicity was not sufficient to mediate a signal in the functional assay measuring NMDAR-mediated calcium flux, while protection of the cells with selected antagonists of the glycine binding and glutamate binding sites facilitated NMDAR function.

EXAMPLE VI

Baculovirus mediated expression of NMDAR in combination with antagonist protection facilitates the sensitivity of NMDAR to ligands of both the glycine binding site and the glutamate binding site

- 5 **[0116]** We then tested whether those antagonists, when washed out, would also render NMDAR more sensitive to exogenously added ligand. In such model, the antagonist would prevent the endogenous ligand from binding to the channel. Once the antagonist is washed out, the channel remains in its ligand-free form and would hence be susceptible to the addition of external ligand.
- 10 **[0117]** We transduced HEK293 cells with NR1/2A and protected the cells with MDL105,519 or CGP070667. After sixteen hours, the protection compounds (*i.e.*, NMDAR ligand binding site antagonists) were washed out and NMDAR-mediated calcium flux was measured on the FDSS after addition of varying concentrations of glycine alone, glutamate alone or a combination of both ligands.
- 15 **[0118]** We transduced HEK293 cells with NR1/2A or NR1/2B baculovirus in the presence of MDL105,519. NMDAR-mediated calcium flux was measured as previously described, after addition of the indicated concentration of glycine and glutamate. Data in TABLE 13 represent the mean of the maximal fluorescence ratio $\pm$ standard deviation (STDEV) of a representative experiment.
- 20 **[0119]** As shown in TABLE 13, MDL105,519-protected cells showed a dose-dependent activation by glycine with an $EC_{50} \sim 12 \mu M$. The transduced HEK293 cells did not show sensitivity to stimulation with glutamate and addition of a saturating concentration of glutamate did not change the EC_{50} for stimulation with glycine. Similarly, protection of the cells with CGP070667, resulted in a dose-dependent sensitivity to glutamate ($EC_{50} \sim 10$
- 25 μM), while glycine alone had no effect.

TABLE 13
Sensitivity of NMDAR-Mediated Calcium Flux in
Protected HEK293 Cells

<u>MDL105,519 protected NR1/2A</u>				
<u>concentration ligand</u> <u>(μM)</u>	<u>100 μM L-glutamate + glycine</u> <u>gradient</u>		<u>100 μM glycine + L-glutamate</u> <u>gradient</u>	
100.00	1.86	1.90	1.94	1.96
33.33	1.72	1.88	1.96	1.78
11.11	1.51	1.59	1.84	1.95
3.70	1.29	1.37	1.83	1.91
1.23	1.19	1.24	1.84	1.83
0.41	1.13	1.17	1.81	1.75
0.14	1.12	1.12	1.85	1.75
0.05	1.16	1.11	1.67	1.83
<u>concentration ligand</u> <u>(μM)</u>	<u>L-glutamate gradient only</u>		<u>glycine gradient only</u>	
100.00	1.12	1.13	1.94	1.79
33.33	1.12	1.15	1.70	1.71
11.11	1.12	1.14	1.49	1.47
3.70	1.08	1.09	1.30	1.26
1.23	1.09	1.09	1.19	1.15
0.41	1.07	1.09	1.08	1.09
0.14	1.04	1.07	1.09	1.11
0.05	1.05	1.04	1.04	1.05
<u>MDL105,519 protected NR1/2B</u>				
	<u>100 μM L-glutamate +</u> <u>glycine gradient</u>		<u>100 μM glycine + L-glutamate</u> <u>gradient</u>	
100.00	1.40	1.45	1.40	1.43
33.33	1.37	1.43	1.46	1.47
11.11	1.18	1.34	1.47	1.43
3.70	1.16	1.18	1.44	1.46
1.23	1.13	1.20	1.47	1.45
0.41	1.13	1.15	1.43	1.52
0.14	1.13	1.12	1.45	1.42
0.05	1.09	1.09	1.41	1.45
	<u>L-glutamate gradient only</u>		<u>glycine gradient only</u>	
100.00	1.09	1.12	1.43	1.39
33.33	1.10	1.11	1.36	1.34
11.11	1.11	1.13	1.37	1.22
3.70	1.09	1.09	1.17	1.19
1.23	1.11	1.09	1.21	1.15
0.41	1.11	1.11	1.15	1.16
0.14	1.13	1.13	1.13	1.14
0.05	1.11	1.09	1.10	1.10
<u>CGP070667 protected NR1/2A</u>				
	<u>100 μM L-glutamate / glycine gradient</u>			
300.00	1.88	1.83	1.94	1.88
100.00	1.95	1.79	1.91	1.78
33.33	1.89	1.72	1.77	1.68
11.11	1.89	1.83	1.87	1.81
3.70	1.96	1.78	1.93	1.72
1.23	1.83	1.82	1.86	1.94
0.41	1.83	1.69	1.66	1.69
0.14	1.90	1.82	1.77	1.77

TABLE 13
Sensitivity of NMDAR-Mediated Calcium Flux in
Protected HEK293 Cells

		<u>100 μM glycine + L-glutamate gradient</u>			
	300.00	1.95	1.98	1.96	1.88
	100.00	1.86	1.80	1.88	1.77
	33.33	1.57	1.50	1.48	1.44
	11.11	1.25	1.31	1.25	1.27
	3.70	1.19	1.18	1.16	1.12
	1.23	1.13	1.09	1.08	1.08
	0.41	1.11	1.09	1.09	1.03
	0.14	1.08	1.11	1.05	1.04
<u>concentration ligand</u>		<u>L-glutamate gradient only</u>			
<u>(μM)</u>					
	300.00	1.52	1.54	1.56	1.68
	100.00	1.59	1.51	1.59	1.56
	33.33	1.30	1.29	1.39	1.26
	11.11	1.16	1.14	1.18	1.22
	3.70	1.08	1.17	1.10	1.08
	1.23	1.04	1.04	1.04	1.05
	0.41	1.02	1.01	1.00	1.02
	0.14	1.01	1.00	1.00	1.00
		<u>glycine gradient only</u>			
	300.00	1.00	1.00	1.00	1.02
	100.00	1.00	1.01	1.02	1.00
	33.33	1.04	1.03	1.03	1.01
	11.11	1.06	1.06	1.03	1.00
	3.70	1.00	1.00	1.00	1.00
	1.23	1.02	1.01	1.01	1.02
	0.41	1.00	1.00	1.00	1.03
	0.14	1.01	1.01	1.00	1.03
<u>CGP070667 protected NR1/2B</u>					
		<u>100 μM L-glutamate / glycine gradient</u>			
	300.00	2.34	2.11	2.00	2.02
	100.00	2.25	2.15	2.04	2.03
	33.33	2.03	1.77	2.05	1.99
	11.11	2.29	1.95	2.03	1.96
	3.70	2.26	2.08	2.06	2.01
	1.23	2.12	2.02	2.01	1.94
	0.41	2.03	2.07	2.03	2.03
	0.14	2.00	2.13	2.23	2.20
		<u>100 μM glycine + L- glutamate gradient</u>			
	300.00	2.07	2.08	2.16	2.22
	100.00	1.80	2.03	2.00	2.06
	33.33	1.81	1.71	1.68	1.87
	11.11	1.65	1.46	1.56	1.61
	3.70	1.46	1.49	1.45	1.46
	1.23	1.31	1.26	1.24	1.28
	0.41	1.25	1.27	1.23	1.19
	0.14	1.25	1.23	1.24	1.23
		<u>L-glutamate gradient only</u>			
	300.00	2.03	2.05	2.06	2.15
	100.00	1.92	1.96	2.04	1.93
	33.33	1.79	1.78	1.83	1.78
	11.11	1.54	1.62	1.64	1.61
	3.70	1.39	1.38	1.38	1.40
	1.23	1.31	1.30	1.28	1.26
	0.41	1.16	1.20	1.21	1.18
	0.14	1.19	1.20	1.18	1.19

TABLE 13					
Sensitivity of NMDAR-Mediated Calcium Flux in Protected HEK293 Cells					
concentration ligand (μ M)		glycine gradient only			
300.00	1.13	1.16	1.15		1.17
100.00	1.12	1.13	1.14		1.12
33.33	1.13	1.11	1.12		1.11
11.11	1.10	1.08	1.10		1.11
3.70	1.12	1.10	1.13		1.16
1.23	1.12	1.17	1.11		1.16
0.41	1.14	1.13	1.16		1.19
0.14	1.17	1.19	1.19		1.18

Data and concentrations were rounded to two decimal places

- [0120]** Glutamate sensitivity of NMDAR-mediated calcium flux in CGP070667 protected cells. HEK293 cells were transduced with NR1/2A or NR1/2B baculovirus in the presence of CGP070667 and NMDAR-mediated calcium flux was analyzed.
- 5 **[0121]** Addition of glycine in addition to glutamate further increased the signal, but did not affect the EC_{50} . These data support our model, in which the antagonists protect the ligand binding site from endogenous ligand in the cell media and, once washed out, increase the percentage of ligand-free receptor on the cell membrane, which can subsequently be activated by the addition of exogenous ligand.
- 10 **[0122]** See, FIG. 3, which illustrates the method of action of the invention. MDL105,519 confers sensitivity to glycine while the glutamate binding site is still occupied by endogenous ligand, explaining the lack of effect of glutamate. On the other hand, CGP070667 keeps the glutamate binding site protected and, when washed out, confers sensitivity to glutamate.

15

EXAMPLE VII

Baculovirus mediated expression of NMDAR shows the expected biology

- [0123]** Similar results were obtained with NMDA and D-serine, while L-serine showed a 200-fold/100-fold (NR1/2A and NR1/2B, respectively) right shift of potency as compared to D-serine. See TABLE 14.

- 20 **[0124]** Sensitivity of NMDAR-mediated calcium flux to D-serine and L-serine. HEK293 cells were transduced with baculovirus encoding for NR1 and NR2A or NR2B in the presence of MDL105,519. NMDAR-mediated calcium flux was measured as previously described in the presence of varying amounts of D-serine or L-serine and saturating
- 25 concentration of glutamate. Data in TABLE 14 represent the mean $\pm$ standard deviation (STDEV) of a representative experiment.

TABLE 14
Sensitivity of NMDAR-Mediated Calcium Flux to D-serine and L-serine

HEK BacMam NR1/2A (F_{max}/F_0)								
<u>concentration</u> <u>ligand (μM)</u>	<u>100 μM L-glutamine/ D-serine</u> <u>gradient</u>				<u>100 μM D-serine /L-glutamine</u> <u>gradient</u>			
0.05	1.10	1.12	1.04	1.13	2.08	1.95	1.95	2.04
0.14	1.15	1.13	1.15	1.07	2.10	2.03	2.08	1.98
0.41	1.28	1.21	1.17	1.16	2.13	2.00	1.95	1.98
1.23	1.26	1.30	1.26	1.27	2.13	1.97	2.11	2.01
3.70	1.53	1.66	1.46	1.33	2.08	2.01	2.08	2.03
5.00								
11.11	1.84	1.82	1.76	1.78	2.11	2.14	2.07	2.16
14.00								
33.33	2.03	2.1	2.02	2.13	2.18	2.19	2.16	2.04
41.00								
100.00	2.3	2.28	2.22	2.16	2.2	2.19	2.12	2.06
123.00								
370.00								
1111.00								
3333.00								
10000.00								
	<u>100 μM L-glutamine/L-serine</u> <u>gradient</u>				<u>D-serine gradient</u>			
0.05					1.03	1.04	1.06	1.05
0.14					1.03	1.03	1.07	1.07
0.41					1.05	1.07	1.08	1.09
1.23					1.15	1.14	1.17	1.15
3.70					1.32	1.34	1.31	1.36
5.00	1.12	1.11	1.13	1.16				
11.11					1.65	1.64	1.54	1.57
14.00	1.15	1.12	1.12	1.16				
33.33					1.9	1.92	1.81	1.84
41.00	1.16	1.14	1.15	1.15				
100.00					2.06	2.06	1.89	1.99
123.00	1.19	1.21	1.19	1.18				
370.00	1.33	1.28	1.31	1.3				
1111.00	1.55	1.57	1.54	1.49				
3333.00	1.99	2.01	1.82	1.79				
10000.00	2.39	2.32	2.14	2.19				
<u>concentration</u> <u>ligand (μM)</u>	<u>L-serine gradient</u>				<u>10 mM L-serine/D-serine gradient</u>			
0.05					1.71	1.73	1.94	1.88
0.14					1.75	1.74	1.89	1.81
0.41					1.7	1.76	1.86	1.79
1.23					1.81	1.79	1.84	1.86
3.70					1.76	1.82	1.72	1.83
5.00	1.02	1.02	1.06	1.07				
11.11					1.9	1.93	1.79	1.79
14.00	1.04	1.04	1.04	1.05				
33.33					1.91	1.88	1.78	1.84
41.00	1.01	1.04	1.05	1.05				
100.00					1.95	1.97	1.79	1.85
123.00	1.07	1.03	1.08	1.08				
370.00	1.11	1.14	1.13	1.13				
1111.00	1.37	1.38	1.29	1.33				
3333.00	1.69	1.7	1.65	1.61				
10000.00	1.85	1.94	1.77	1.79				

TABLE 14
Sensitivity of NMDAR-Mediated Calcium Flux to D-serine and L-serine

HEK BacMam NR1/2B (F_{max}/F_0)								
concentration ligand (μ M)	100 μ M L-glutamine/D-serine gradient				100 μ M D-serine/L-glutamine gradient			
0.05	1.10	1.10	1.11	1.09	1.50	1.56	1.54	1.63
0.14	1.11	1.11	1.13	1.13	1.55	1.52	1.61	1.58
0.41	1.16	1.17	1.14	1.15	1.58	1.62	1.64	1.63
1.23	1.21	1.18	1.2	1.21	1.54	1.56	1.57	1.58
3.70	1.29	1.27	1.31	1.30	1.59	1.56	1.61	1.62
5.00								
11.11	1.50	1.49	1.45	1.44	1.62	1.58	1.56	1.64
14.00								
33.33	1.59	1.55	1.52	1.51	1.64	1.62	1.57	1.55
41.00								
100.00	1.68	1.65	1.63	1.54	1.67	1.67	1.59	1.57
123.00								
370.00								
1111.00								
3333.00								
10000.00								
concentration ligand (μ M)	100 μ M L-glutamine/L-serine gradient				D-serine gradient			
0.05					1.07	1.06	1.07	1.05
0.14					1.09	1.06	1.08	1.07
0.41					1.09	1.11	1.11	1.13
1.23					1.17	1.18	1.18	1.19
3.70					1.29	1.27	1.28	1.29
5.00	1.12	1.09	1.07	1.07				
11.11					1.46	1.46	1.39	1.44
14.00	1.10	1.11	1.10	1.09				
33.33					1.52	1.51	1.51	1.49
41.00	1.13	1.13	1.12	1.11				
100.00					1.62	1.61	1.51	1.56
123.00	1.16	1.15	1.16	1.12				
370.00	1.22	1.25	1.25	1.24				
1111.00	1.22	1.39	1.46	1.42				
3333.00	1.57	1.47	1.57	1.52				
10000.00	1.49	1.49	1.56	1.52				
concentration ligand (μ M)	L-serine gradient				10 mM L-serine/D-serine gradient			
0.05					1.42	1.45	1.52	1.52
0.14					1.45	1.49	1.48	1.51
0.41					1.44	1.54	1.45	1.55
1.23					1.46	1.50	1.47	1.43
3.70					1.44	1.50	1.49	1.45
5.00	1.09	1.10	1.06	1.08				
11.11					1.49	1.49	1.47	1.50
14.00	1.05	1.08	1.07	1.06				
33.33					1.46	1.50	1.45	1.40
41.00	1.10	1.09	1.09	1.09				
100.00					1.47	1.39	1.46	1.53
123.00	1.12	1.11	1.10	1.12				
370.00	1.31	1.20	1.19	1.20				
1111.00	1.31	1.35	1.35	1.34				
3333.00	1.47	1.47	1.49	1.47				
10000.00	1.44	1.38	1.48	1.50				

Data and concentrations were rounded two decimal places

[0125] The IC_{50} of Mg^{2+} decreases with lower membrane potential. The driving force for calcium increases with lower membrane potential, but the channel is blocked by magnesium.

[0126] Taken together, our data demonstrate the utility of the cellular, plate-based assay of the invention, which enables neurobiologists and biochemists to study both agonist-dependent and co-agonist-dependent NMDAR activity.

[0127] The method of the invention can use an NMDAR ligand binding site antagonist to protect the NMDAR ligand binding site from endogenous ligand. This is not limited only to baculovirus mediated expression of NMDAR, but can also be used with NMDAR stably expressed under the control of an inducible promotor highlighting the general applicability of our approach. The data presented in TABLE 15 show HEK293 cells stably expressing NR1 and NR2A under the control for a tetracycline-inducible promotor. These HEK293 cells were seeded and induced with tetracycline in the presence of MDL105.519 or CGP070667 and NMDAR-mediated calcium flux was measured as previously described after addition of the indicated concentration of glycine and glutamate. Data in TABLE 15 represent the mean of the maximal fluorescence ratio of a representative experiment.

TABLE 15
Baculovirus Mediated Expression of NMDAR Shows Expected Biology

<u>MDL105,519 protected NR1/2A</u>						
<u>Concentration</u>	<u>D-serine only</u>					
<u>ligand (μM)</u>						
300.00	6.66	5.51	4.43	4.26	3.28	3.40
100.00	4.45	3.95	4.02	2.81	2.21	2.73
33.33	3.10	2.84	2.79	2.31	2.19	1.81
11.11	2.04	1.99	1.87	1.48	1.37	1.49
3.70	1.21	1.20	1.32	1.08	1.14	1.22
1.23	1.07	1.08	1.07	1.06	1.05	1.02
0.41	1.03	1.00	0.99	1.04	1.02	1.03
0.14	1.03	0.99	0.99	1.06	1.05	1.01
	<u>glutamate only</u>					
300.00	3.16	3.77	3.51	1.72	1.75	1.85
100.00	2.29	2.34	2.38	1.80	1.90	1.84
33.33	2.13	2.06	2.04	1.46	1.58	1.45
11.11	1.85	1.90	1.72	1.42	1.57	1.48
3.70	1.64	1.78	1.67	1.39	1.52	1.47
1.23	1.30	1.32	1.35	1.42	1.40	1.47
0.41	1.11	1.10	1.11	1.22	1.17	1.21
0.14	1.01	1.01	1.00	1.10	1.08	1.10
	<u>glutamate + D-serine gradient</u>					
300.00	6.29	5.02	6.15	3.79	4.72	3.59
100.00	5.71	4.83	6.70	3.99	4.53	3.27
33.33	3.89	3.44	3.85	3.29	3.32	2.78
11.11	3.20	3.17	3.14	2.27	2.70	2.06
3.70	2.75	3.62	2.89	2.66	2.85	4.87
1.23	2.56	2.28	1.97	2.53	2.99	2.86
0.41	2.17	2.14	1.13	2.99	3.10	3.19

TABLE 15
Baculovirus Mediated Expression of NMDAR Shows Expected Biology

	0.14	1.89	1.87	1.49	2.85	3.13	2.90
	<u>D-serine + glutamate gradient</u>						
300.00	5.62	6.72	5.62	4.32	3.83	3.63	
100.00	4.49	4.17	4.49	4.36	3.65	3.43	
33.33	4.33	4.31	5.41	3.31	3.44	3.21	
11.11	3.85	3.68	4.34	3.14	3.61	2.82	
3.70	3.40	3.70	3.80	3.55	3.55	3.99	
1.23	3.67	3.15	3.18	3.52	3.59	3.61	
0.41	3.74	3.26	3.08	4.85	4.20	2.82	
0.14	2.78	2.85	2.92	4.60	4.46	2.06	
	<u>CGP070667 protected NR1/2A</u>						
	<u>D-serine only</u>						
300.00	1.08	1.08	1.08	1.02	1.02	1.03	
100.00	1.00	1.03	1.05	1.01	1.00	1.02	
33.33	1.03	1.00	1.03	0.98	0.98	0.98	
11.11	1.00	1.00	1.02	0.98	0.98	0.98	
3.70	0.98	0.98	1.00	0.99	0.99	0.98	
1.23	0.99	0.99	0.99	0.98	0.98	0.98	
0.41	0.99	0.97	0.98	1.01	1.00	1.00	
0.14	0.99	0.98	0.97	1.03	1.00	1.01	
<u>Concentration</u>	<u>glutamate only</u>						
<u>ligand (μM)</u>							
300.00	6.38	6.81	7.42	3.49	3.62	3.47	
100.00	4.39	6.12	5.63	3.36	3.47	3.58	
33.33	4.55	4.71	4.69	3.02	3.34	2.95	
11.11	3.09	3.95	3.34	2.58	2.61	2.62	
3.70	2.77	3.04	2.88	2.38	2.57	2.71	
1.23	1.83	1.87	2.00	2.02	2.22	2.04	
0.41	1.27	1.30	1.26	1.34	1.59	1.43	
0.14	1.05	1.07	1.04	1.09	1.13	1.11	
	<u>glutamate + D-serine gradient</u>						
300.00	6.25	7.86	6.58	3.91	3.64	3.92	
100.00	6.53	5.50	4.90	3.46	3.83	3.46	
33.33	4.61	5.07	4.74	3.27	3.28	3.28	
11.11	4.19	4.62	4.77	3.12	3.01	3.08	
3.70	4.29	4.30	4.10	3.84	3.64	3.46	
1.23	3.55	4.00	3.36	3.78	3.77	3.49	
0.41	3.58	3.63	6.30	4.92	3.56	4.09	
0.14	3.34	4.03	3.36	5.60	5.85	4.77	
	<u>D-serine + glutamate gradient</u>						
300.00	7.69	11.03	10.35	4.79	4.53	4.72	
100.00	5.17	6.14	7.58	4.31	4.74	4.76	
33.33	4.87	6.26	6.92	3.32	4.14	3.60	
11.11	4.36	5.22	5.45	3.10	3.78	3.78	
3.70	3.79	3.23	4.73	3.27	3.22	4.17	
1.23	2.95	2.65	2.89	2.56	2.79	3.63	
0.41	2.43	2.03	2.01	2.15	2.31	3.26	
0.14	1.28	1.36	1.29	1.51	1.54	2.17	

Data and concentrations rounded to two decimal places

EXAMPLE VIII

The dual ligand sensitivity of NMDAR after antagonist protection can be used to characterize the mode of action of NMDAR inhibitors

[0128] Having shown the dual ligand sensitivity of NMDAR after antagonist protection,
5 we used our assay of the invention to dissect the mechanism of action of NMDAR inhibitors. We hypothesized that exogenously added D-serine competes with the effect of the glycine binding site antagonist MDL105,519 and is hence expected to result in a right shift of the dose response curve. Similarly, addition of glutamate should right shift the dose response curve of a glutamate binding site antagonist. Neither ligand was expected
10 to show an effect on potency of the channel blocker MK801.

[0129] We transduced HEK293 cells with NR1/2A, protected with MDL105,519 or CGP070667 as indicated and measured NMDAR activity in the presence of varying concentrations of MDL105,519, CGP070667 or MK801 after stimulation with either the EC₂₀, EC₄₀, EC₆₀ and EC₁₀₀ of D-serine or glutamate with saturating concentration of the
15 co-agonist (100 μ M). Stimulation of D-serine sensitive cells with the EC₁₀₀ of D-serine caused a significant right shift of the dose response curve for MDL105,519 as compared to the EC₂₀ of D-serine, while there was little effect on the potency of CGP070667 or MK801. These data are in line with the expected effect of a glycine binding site inhibitor.

[0130] Conversely, CGP070667 showed a glutamate dependent shift in potency in
20 glutamate-sensitive cells, while the concentration of glutamate had only minor effects on the potency of MDL105,519 or MK801 in these cells. These data are in line with the expected effect of a glutamate-binding site inhibitor, while the observed independence of the potency of MK801 on either ligand concentration points to a negative allosteric modulator or channel blocker. We obtained similar results for NR1/2B.

[0131] TABLE 16 shows the sensitivity of NR1/2A-mediated or NR1/2B-mediated
25 calcium flux to inhibition by CGP070667, MDL105,519 or MK801 at different ligand concentrations. HEK293 cells were transduced with baculovirus vector encoding NR1 and NR2A or NR2B in the presence of MDL105,519 or CGP070667 and NMDAR-mediated calcium flux was measured as previously described in the presence of the
30 indicated ligand concentrations. The integral of the maximal fluorescence ratio was normalized to the activity measured in the absence of inhibitor (DMSO only) at the respective ligand concentration. Data represents the mean of a representative experiment.

TABLE 16
Sensitivity of NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux to Inhibition

MDL105,519 protected

<u>EC₂₀ D-serine</u>				<u>MDL105,519</u>				<u>MK801</u>	
	<u>CGP070667</u>								
30.00	0.13	0.09	0.09	0.19	0.18	0.15	0.06	0.14	0.10
10.00	0.17	0.28	0.14	0.11	-0.01	0.09	0.07	-0.05	0.09
3.33	-0.01	-0.09	0.02	-0.01	-0.15	-0.11	-0.05	-0.11	-0.03
1.11	-0.05	-0.18	-0.09	0.06	-0.03	-0.12	-0.08	-0.08	-0.05
0.37	-0.02	-0.05	0.01	-0.01	-0.05	-0.02	-0.12	-0.08	0.02
0.12	-0.02	0.05	0.03	0.29	0.16	0.16	0.13	0.12	0.22
0.04	-0.04	-0.04	0.21	0.32	0.30	0.30	0.22	0.08	0.18
0.01	0.54	0.27	0.12	0.53	0.66	0.59	0.37	0.29	0.28

<u>EC₄₀ D-serine</u>				<u>MDL105,519</u>				<u>MK801</u>	
	<u>CGP070667</u>								
30.00	0.15	0.12	0.08	0.03	0.09	0.08	0.08	0.16	0.14
10.00	0.20	0.16	0.18	0.08	0.02	-0.01	0.07	-0.03	0.02
3.33	0.16	0.00	0.01	0.03	-0.15	-0.04	-0.10	-0.18	-0.08
1.11	0.14	0.12	0.09	0.02	-0.11	-0.10	-0.14	-0.06	-0.07
0.37	0.07	0.07	0.01	0.08	0.05	0.12	-0.19	-0.15	-0.04
0.12	0.08	0.12	-0.04	0.39	0.50	0.30	0.07	0.10	0.15
0.04	0.22	0.10	0.06	0.49	0.58	0.54	0.25	0.12	0.23
0.01	0.28	0.33	0.18	0.66	0.63	0.71	0.31	0.36	0.50

<u>EC₈₀ D-serine</u>				<u>MDL105,519</u>				<u>MK801</u>	
	<u>CGP070667</u>								
30.00	-0.01	0.04	0.01	-0.12	-0.04	0.01	-0.02	-0.07	-0.04
10.00	0.04	0.04	0.11	-0.06	-0.03	-0.03	-0.04	-0.03	-0.01
3.33	0.09	0.06	0.01	-0.07	-0.04	-0.07	-0.13	-0.09	-0.05
1.11	0.07	0.08	0.05	0.14	0.13	0.12	-0.08	-0.09	-0.02
0.37	0.08	0.07	0.00	0.26	0.41	0.48	-0.04	0.07	-0.01
0.12	0.15	0.14	0.12	0.55	0.66	0.81	0.12	0.19	0.20
0.04	0.21	0.19	0.20	0.42	1.02	0.63	0.40	0.54	0.32
0.01	0.18	0.16	0.19	0.50	0.73	0.87	0.30	0.64	0.52

<u>EC₁₀₀ D-serine</u>				<u>MDL105,519</u>				<u>MK801</u>	
	<u>CGP070667</u>								
30.00	-0.05	-0.03	-0.10	-0.11	-0.06	-0.08	-0.04	-0.03	-0.05
10.00	-0.06	0.01	0.00	-0.04	-0.02	-0.03	-0.05	-0.03	-0.03
3.33	-0.01	-0.03	-0.09	0.14	0.05	0.07	-0.07	-0.08	-0.04
1.11	0.08	0.05	-0.01	0.60	0.42	0.36	-0.06	0.01	0.00
0.37	0.07	0.04	0.04	0.74	1.04	0.82	0.02	-0.03	0.07
0.12	0.16	0.38	0.29	0.94	1.13	0.94	0.22	0.25	0.19
0.04	0.35	0.45	0.17	0.86	0.77	0.81	0.62	0.37	0.32
0.01	0.22	0.44	0.68	0.97	0.97	1.00	0.59	0.70	0.54

CGP070667 protected NR1/2A

<u>EC₂₀ glutamate</u>				<u>MDL105,519</u>				<u>MK801</u>	
	<u>CGP070667</u>								
30.00	0.06	0.03	0.03	0.04	0.02	0.01	0.01	0.01	-0.05
10.00	0.13	0.03	0.05	0.02	-0.08	-0.05	0.01	-0.04	-0.01
3.33	0.06	-0.03	0.01	0.04	-0.03	-0.04	-0.03	-0.07	-0.08
1.11	0.00	0.04	0.01	0.23	0.18	0.13	0.01	-0.05	-0.02
0.37	0.13	0.02	0.08	0.36	0.30	0.31	0.11	0.09	0.17
0.12	0.54	0.09	0.20	0.49	0.41	0.41	0.34	0.34	0.36
0.04	0.53	0.34	0.38	0.52	0.54	0.79	0.53	0.47	0.52
0.01	0.60	0.52	0.52	0.60	0.55	0.60	0.60	0.59	0.64

TABLE 16
Sensitivity of NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux to Inhibition

<u>EC₄₀ glutamate</u>									
	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	0.01	-0.05	-0.02	-0.02	-0.04	-0.04	-0.01	-0.04	-0.07
10.00	0.04	0.00	0.01	-0.02	-0.05	-0.08	0.03	-0.06	-0.06
3.33	-0.02	-0.01	-0.02	0.03	-0.01	0.00	-0.03	-0.07	-0.07
1.11	-0.02	0.01	0.03	0.13	0.09	0.10	-0.04	-0.05	-0.05
0.37	0.01	-0.01	0.04	0.31	0.26	0.22	0.08	0.06	0.10
0.12	0.19	0.13	0.10	0.42	0.38	0.47	0.30	0.28	0.30
0.04	0.34	0.23	0.29	0.46	0.46	0.48	0.49	0.45	0.48
0.01	0.50	0.38	0.42	0.60	0.58	0.55	0.55	0.57	0.61
<u>EC₈₀ glutamate</u>									
	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	0.00	0.01	0.00	0.00	0.00	-0.03	0.00	-0.02	-0.01
10.00	0.08	0.03	0.07	0.00	0.02	0.03	-0.03	0.04	-0.05
3.33	-0.01	0.01	0.04	0.05	0.02	0.09	0.00	-0.04	-0.04
1.11	0.05	0.05	0.03	0.23	0.25	0.27	0.03	0.02	0.00
0.37	0.06	0.02	0.02	0.37	0.37	0.59	0.10	0.11	0.11
0.12	0.17	0.18	0.14	0.47	0.53	0.60	0.32	0.30	0.34
0.04	0.40	0.32	0.28	0.55	0.62	0.69	0.52	0.51	0.57
0.01	0.64	0.54	0.51	0.78	0.82	0.82	0.70	0.88	0.77
<u>EC₁₀₀ glutamate</u>									
	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	0.02	0.00	0.04	0.00	0.01	0.02	-0.01	0.00	0.03
10.00	0.06	0.08	0.07	0.08	0.13	0.09	0.03	0.06	0.03
3.33	0.05	0.09	0.07	0.20	0.21	0.26	0.06	0.06	0.05
1.11	0.26	0.24	0.24	0.54	0.39	0.43	0.13	0.21	0.12
0.37	0.44	0.41	0.40	0.51	0.61	0.64	0.23	0.24	0.26
0.12	0.64	0.69	0.65	0.62	0.74	0.70	0.45	0.43	0.40
0.04	0.82	0.78	0.78	0.70	0.71	0.73	0.61	0.64	0.60
0.01	0.96	0.84	0.82	0.78	0.81	0.86	0.69	0.74	0.76
<u>MDL105,519 protected NR1/2B</u>									
<u>EC₂₀ D-serine</u>									
	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	0.25	0.07	0.19	0.07	0.09	0.10	0.14	0.07	0.08
10.00	0.26	0.13	0.16	0.09	-0.01	0.00	0.01	-0.02	0.01
3.33	0.21	0.09	0.12	0.10	-0.09	-0.02	0.02	-0.11	-0.04
1.11	0.19	0.19	0.13	0.12	0.06	0.08	-0.06	-0.06	-0.09
0.37	0.32	0.13	0.17	0.22	0.22	0.24	-0.06	-0.05	-0.07
0.12	0.54	0.28	0.30	0.42	0.49	0.50	0.01	0.05	0.08
0.04	0.44	0.35	0.36	0.47	0.50	0.51	0.09	0.05	0.09
0.01	0.47	0.45	0.54	0.46	0.54	0.66	0.24	0.20	0.18
<u>EC₄₀ D-serine</u>									
	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	-0.03	0.03	0.13	0.08	0.07	0.09	0.09	0.04	0.11
10.00	0.27	0.19	0.13	0.14	0.10	0.05	0.11	0.03	0.10
3.33	0.08	0.09	0.07	0.12	0.03	0.02	-0.07	-0.03	0.01
1.11	0.08	0.16	0.13	0.19	0.11	0.09	0.07	-0.05	0.01
0.37	0.25	0.15	0.10	0.53	0.42	0.34	-0.06	-0.03	-0.04
0.12	0.32	0.44	0.36	0.57	0.70	0.53	-0.01	0.03	0.06
0.04	0.80	0.62	0.73	0.90	0.89	0.72	0.16	0.12	0.18
0.01	0.56	0.64	0.63	0.73	0.71	0.89	0.30	0.24	0.30

TABLE 16
Sensitivity of NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux to Inhibition

<u>EC₈₀ D-serine</u>									
	<u>CGP070667</u>				<u>MDL105,519</u>			<u>MK801</u>	
30.00	-0.02	-0.01	0.03	-0.04	-0.03	-0.04	-0.03	0.02	0.00
10.00	0.04	0.09	0.06	0.03	0.03	0.05	0.07	-0.01	0.04
3.33	0.06	0.02	0.03	0.08	0.07	0.12	-0.02	0.01	-0.02
1.11	0.13	0.16	0.10	0.44	0.36	0.43	-0.01	-0.01	0.06
0.37	0.24	0.24	0.13	0.69	0.66	0.72	-0.04	-0.04	-0.01
0.12	0.40	0.48	0.32	0.93	0.75	0.77	0.10	0.01	0.11
0.04	0.68	0.69	0.47	1.00	1.00	0.87	0.12	0.20	0.12
0.01	0.47	0.55	0.61	0.85	0.80	0.77	0.28	0.36	0.29
<u>EC₁₀₀ D-serine</u>									
	<u>CGP070667</u>				<u>MDL105,519</u>			<u>MK801</u>	
30.00	0.08	0.11	0.04	-0.04	0.00	0.01	0.01	0.01	0.02
10.00	0.05	0.04	0.11	0.09	0.15	0.18	0.01	-0.01	-0.03
3.33	0.07	0.09	0.12	0.50	0.31	0.45	-0.04	-0.02	-0.03
1.11	0.19	0.28	0.31	0.68	0.53	0.54	-0.01	-0.01	0.01
0.37	0.49	0.50	0.39	0.75	0.82	0.81	-0.05	0.01	0.04
0.12	0.73	0.96	0.81	0.86	0.77	0.88	-0.02	-0.03	0.03
0.04	1.00	0.79	0.64	0.79	0.86	0.84	0.07	0.11	0.14
0.01	0.98	0.93	1.04	0.80	0.84	0.94	0.28	0.38	0.39
<u>CGP070667 protected NR1/2B</u>									
<u>EC₂₀ glutamate</u>									
	<u>CGP070667</u>				<u>MDL105,519</u>			<u>MK801</u>	
30.00	0.00	0.00	0.00	0.09	0.09	0.05	0.02	0.02	-0.01
10.00	0.03	0.02	0.01	0.12	0.00	0.07	0.00	-0.03	-0.04
3.33	-0.02	-0.03	-0.02	0.41	0.17	0.25	-0.06	-0.11	-0.10
1.11	0.01	0.01	0.05	0.56	0.48	0.45	-0.06	-0.08	-0.04
0.37	0.18	0.42	0.25	0.71	0.52	0.55	-0.08	-0.06	-0.05
0.12	0.41	0.37	0.37	0.59	0.53	0.62	0.15	0.08	0.17
0.04	0.52	0.49	0.48	0.79	0.77	0.61	0.38	0.34	0.36
0.01	0.61	0.76	0.76	0.74	0.76	0.76	0.58	0.56	0.66
<u>EC₄₀ glutamate</u>									
	<u>CGP070667</u>				<u>MDL105,519</u>			<u>MK801</u>	
30.00	0.12	0.05	0.10	0.14	0.10	0.18	0.08	0.05	0.09
10.00	0.05	0.11	0.08	0.14	0.11	0.12	0.14	0.05	0.06
3.33	0.01	0.11	0.02	0.21	0.20	0.25	0.03	0.03	0.03
1.11	0.10	0.08	0.13	0.40	0.38	0.43	0.00	0.00	0.04
0.37	0.20	0.23	0.23	0.69	0.58	0.46	0.04	-0.03	-0.02
0.12	0.53	0.46	0.41	0.57	0.68	0.65	0.20	0.09	0.12
0.04	0.60	0.63	0.64	0.75	0.84	0.72	0.42	0.37	0.44
0.01	0.62	0.74	0.64	0.75	0.66	0.83	0.60	0.57	0.62
<u>EC₈₀ glutamate</u>									
	<u>CGP070667</u>				<u>MDL105,519</u>			<u>MK801</u>	
30.00	0.06	0.08	0.11	0.12	0.05	0.07	0.03	0.05	0.04
10.00	0.07	0.14	0.13	0.23	0.19	0.29	0.03	0.05	0.06
3.33	0.13	0.15	0.11	0.37	0.41	0.45	0.01	0.01	0.03
1.11	0.16	0.21	0.22	0.61	0.63	0.60	0.00	0.03	0.06
0.37	0.35	0.29	0.33	0.65	0.57	0.82	0.02	0.08	0.11
0.12	0.60	0.57	0.51	0.70	0.69	0.82	0.17	0.18	0.26
0.04	0.73	0.68	0.60	0.89	0.80	0.92	0.40	0.38	0.49
0.01	0.76	0.80	0.79	0.83	0.75	0.93	0.57	0.67	0.67

TABLE 16
Sensitivity of NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux to Inhibition

<u>EC₁₀₀ glutamate</u>	<u>CGP070667</u>			<u>MDL105,519</u>			<u>MK801</u>		
30.00	0.01	0.01	0.04	0.05	0.16	0.02	-0.03	-0.03	-0.04
10.00	0.17	0.24	0.06	0.41	0.42	0.38	-0.04	0.00	-0.02
3.33	0.17	0.49	0.29	0.41	0.52	0.61	-0.04	-0.04	-0.02
1.11	0.65	0.51	0.49	0.76	0.61	0.74	-0.01	-0.04	-0.04
0.37	0.84	0.88	0.84	0.69	0.87	0.79	0.03	0.03	0.04
0.12	0.84	1.02	0.86	0.83	0.84	0.82	0.20	0.16	0.16
0.04	0.89	0.92	0.91	0.85	0.92	0.88	0.47	0.43	0.43
0.01	1.21	1.08	0.99	0.95	1.01	1.04	0.60	0.68	0.71

Data and concentrations were rounded to two decimal places

[0132] These results show the ability of our dual ligand-sensitive NMDAR assay to dissect the mechanism of action of NMDAR inhibitors.

EXAMPLE IX

- 5 *The dual ligand sensitive system for NMDAR can be used to characterize the mode of action of positive allosteric modulators of NMDAR*

[0133] Having shown the applicability of the assay of the invention to identify inhibitors of NMDAR and to characterize their mechanism of action, we tested whether our system would also identify positive allosteric modulators (PAMs) of NMDAR.

- 10 **[0134]** For this, we transduced HEK293 cells with NR1/2A or NR1/2B and protected the cells with MDL105,519 or CGP070667. After sixteen hours, the protection compound (*i.e.*, the NMDAR ligand binding site antagonist) was washed out. NMDAR-mediated calcium flux was measured on the FDSS after addition of varying concentrations of known NMDAR PAMs, followed by addition of ligand. Taking advantage of the dual
- 15 ligand-sensitivity of our system, we measured the effect of the positive allosteric modulators at the EC₃₀ of either ligand in the presence of saturating amount of the other ligand, as well as in the presence of saturating amounts of both ligands. This allowed us to assess the ligand-dependent as well as the ligand-independent effect of the PAMs, respectively.

- 20 **[0135]** TABLE 17 shows the effect of positive allosteric modulators of NMDAR on NR1/2A-mediated or NR1/2B-mediated calcium flux. We transduced HEK293 cells with baculovirus encoding for NR1 protein and either NR2A or NR2B proteins in the presence of MDL105,519 or CGP070667. We then measured NMDAR-mediated calcium flux (as described above) in the presence of the indicated ligand concentrations. Cells were
- 25 incubated with the indicated concentrations of the ligands spermine, pregnenolone sulfate (PS), CIQ or GNE-6901 for five minutes before addition of NMDAR ligand site antagonist.

[0136] TABLE 17 shows the relative activity measured before the addition of ligand (normalized to DMSO-treated cells), as compared with the relative activity measured after addition of the indicated concentration of ligand. The data in TABLE 17 were normalized to the activity in the presence of saturating amounts of both ligands in the presence of DMSO. The data represents the mean of a representative experiment.

TABLE 17						
Effect of NMDAR Positive Allosteric Modulators						
on NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux						
GNE-6901						
NR1/2A						
log conc. compound	NR2A - EC ₃₀ D-serine			NR2A - EC ₁₀₀ MDL105,519		
2.00	0.95	1.15	0.97	1.35	1.47	1.61
1.52	1.17	0.99	1.02	1.44	1.59	1.60
1.05	0.85	0.92	0.99	1.56	1.53	1.44
0.57	0.86	0.72	0.74	1.72	1.77	1.64
0.09	0.58	0.58	0.53	1.39	1.50	1.46
-0.39	0.30	0.31	0.36	1.07	1.21	1.28
-0.86	0.24	0.24	0.26	1.03	1.27	1.12
-1.34	0.23	0.20	0.20	0.80	0.90	1.30
NR2A - EC ₃₀ glutamate						
2.00	1.28	1.29	1.26	1.12	1.31	1.52
1.52	1.36	1.26	1.32	1.28	1.35	1.35
1.05	1.34	1.28	1.20	1.30	1.46	1.63
0.57	1.11	1.24	1.19	1.24	1.60	1.29
0.09	0.90	0.84	0.87	1.18	1.25	1.27
-0.39	0.49	0.61	0.65	0.95	1.06	1.03
-0.86	0.34	0.40	0.41	1.03	1.03	0.99
-1.34	0.24	0.36	0.33	0.92	1.00	1.09
NR1/2B						
NR2A - EC ₃₀ D-serine						
2.00	0.43	0.26	0.31	0.44	0.57	0.82
1.52	0.32	0.54	0.50	0.59	0.95	1.06
1.05	0.25	0.27	0.31	1.12	1.15	1.19
0.57	0.41	0.26	0.29	1.04	1.31	1.40
0.09	0.10	0.29	0.24	1.27	1.17	0.97
-0.39	0.20	0.27	0.21	1.14	1.11	1.10
-0.86	0.25	0.17	-0.02	1.17	1.04	1.03
-1.34	0.36	0.30	0.29	0.60	1.25	1.15
NR2A - EC ₃₀ glutamate						
2.00	0.32	0.25	0.45	0.48	0.63	0.57
1.52	0.30	0.35	0.30	0.72	0.78	0.85
1.05	0.21	0.44	0.30	1.04	0.92	0.90
0.57	0.25	0.38	0.31	1.12	1.29	1.10
0.09	0.29	0.26	0.32	1.01	1.06	0.93
-0.39	0.17	0.34	0.22	0.81	0.85	0.93
-0.86	0.17	0.19	0.26	0.92	1.12	1.04
-1.34	0.31	0.31	0.49	0.89	1.06	1.05

TABLE 17
Effect of NMDAR Positive Allosteric Modulators
on NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux

<u>log conc.</u> <u>compound</u>	<u>1st interval</u>					
	<u>NR2A - MDL105,519</u>			<u>NR2A - CGP070667</u>		
2.00	1.14	1.17	1.16	1.37	1.44	1.37
1.52	1.16	1.15	1.23	1.49	1.39	1.44
1.05	1.09	1.11	1.18	1.45	1.40	1.40
0.57	1.09	1.10	1.08	1.37	1.35	1.39
0.09	1.01	1.00	1.04	1.25	1.23	1.21
-0.39	0.97	1.00	1.01	1.10	1.15	1.12
-0.86	0.94	0.95	0.94	1.02	1.02	1.08
-1.34	0.91	0.91	0.92	1.00	1.00	1.02
	<u>NR2B - MDL105,519</u>			<u>NR2B - CGP070667</u>		
2.00	1.01	0.98	0.99	1.01	1.00	1.04
1.52	0.98	1.00	1.00	1.03	1.03	1.06
1.05	0.99	0.97	0.98	1.01	1.08	1.05
0.57	0.98	0.98	0.99	1.02	1.06	1.05
0.09	0.94	0.97	0.97	1.01	0.99	1.00
-0.39	0.97	0.97	0.97	1.01	1.04	1.00
-0.86	0.98	0.96	0.94	0.98	0.99	1.02
-1.34	0.97	0.97	0.97	0.99	1.02	1.07
	<u>Spermine</u>					
	<u>NR1/2A</u>					
	<u>NR2A - EC₃₀ D-serine</u>			<u>NR2A - EC₁₀₀ MDL105,519</u>		
2.00	0.42	0.47	0.35	1.06	1.09	1.08
1.52	0.33	0.27	0.35	0.85	0.80	1.03
1.05	0.22	0.19	0.26	0.94	0.95	0.87
0.57	0.21	0.21	0.22	0.89	0.70	0.86
0.09	0.22	0.16	0.20	0.81	0.73	0.78
-0.39	0.20	0.21	0.22	0.86	0.77	0.96
-0.86	0.21	0.22	0.21	1.08	0.88	0.85
-1.34	0.18	0.26	0.26	0.86	0.93	1.21
	<u>NR2A - EC₃₀ glutamate</u>			<u>NR2A - EC₁₀₀ CGP070667</u>		
2.00	0.23	0.22	0.31	1.00	1.06	0.93
1.52	0.19	0.25	0.18	0.81	0.80	1.07
1.05	0.12	0.17	0.24	1.04	1.00	0.89
0.57	0.17	0.17	0.18	0.94	0.88	0.95
0.09	0.27	0.21	0.18	0.73	0.94	0.67
-0.39	0.20	0.18	0.24	0.99	0.87	0.96
-0.86	0.18	0.19	0.19	1.08	1.07	1.07
-1.34	0.18	0.21	0.18	0.94	1.02	1.03
	<u>NR1/2B</u>					
	<u>NR2B - EC₃₀ D-serine</u>			<u>NR2B - EC₁₀₀ MDL105,519</u>		
2.00	1.37	1.53	1.34	2.70	3.04	2.99
1.52	0.99	1.11	0.95	1.65	2.23	2.50
1.05	0.57	0.52	0.55	1.51	1.41	1.75
0.57	0.33	0.41	0.40	1.37	0.93	1.28
0.09	0.15	0.26	0.24	1.88	1.35	1.98
-0.39	0.28	0.24	0.20	1.45	1.71	1.66
-0.86	0.23	0.18	0.07	1.36	1.02	1.18
-1.34	0.18	0.51	0.44	0.79	1.06	1.15
	<u>NR2B - EC₃₀ glutamate</u>			<u>NR2B - EC₁₀₀ CGP070667</u>		
2.00	0.42	0.47	0.37	1.18	1.33	1.30
1.52	0.47	0.57	0.41	1.14	0.94	1.52
1.05	0.39	0.29	0.32	0.96	1.19	1.11
0.57	0.31	0.26	0.35	1.12	1.07	0.96
0.09	0.25	0.34	0.30	0.81	1.03	0.92

TABLE 17
Effect of NMDAR Positive Allosteric Modulators
on NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux

-0.39	0.21	0.24	0.35	0.90	0.96	1.02
-0.86	0.13	0.18	0.18	1.09	0.98	1.09
-1.34	0.31	0.26	0.22	1.07	1.03	0.91
<u>1st interval</u>						
<u>NR2A - MDL</u>			<u>NR2A - CGP070667</u>			
2.00	1.01	1.07	0.96	1.01	1.04	1.01
1.52	0.98	0.97	0.99	1.01	0.98	0.99
1.05	0.94	0.92	0.95	0.95	0.96	0.98
0.57	0.93	0.95	0.94	1.00	0.98	0.99
0.09	0.91	0.90	0.93	0.98	0.97	0.96
-0.39	0.92	0.94	0.94	0.99	0.97	1.01
-0.86	0.96	0.94	0.92	0.99	0.97	1.00
-1.34	0.92	0.95	0.96	0.99	0.98	0.97
<u>NR2B - MDL</u>			<u>NR2B - CGP070667</u>			
2.00	1.14	1.12	1.15	1.08	1.09	1.03
1.52	1.09	1.11	1.09	1.06	1.06	1.06
1.05	1.02	1.01	1.03	1.07	1.05	1.06
0.57	0.97	1.00	1.01	1.01	1.01	1.03
0.09	0.96	0.97	0.96	1.00	1.04	1.02
-0.39	0.96	0.98	0.96	1.02	1.03	1.04
-0.86	0.97	0.96	0.95	1.03	1.02	0.99
-1.34	0.97	0.99	0.99	1.04	1.03	1.04
<u>Pregnenolone sulfate</u>						
<u>NR1/2A</u>						
<u>NR2A - EC₃₀ D-Serine</u>			<u>NR2A - EC₁₀₀ MDL105,519</u>			
2.00	0.32	0.40	0.49	0.96	1.22	1.42
1.52	0.46	0.57	0.49	1.32	1.26	1.08
1.05	0.41	0.33	0.40	1.21	1.12	1.21
0.57	0.27	0.28	0.30	0.84	1.22	1.18
0.09	0.17	0.26	0.16	1.06	1.08	1.15
-0.39	0.21	0.23	0.21	1.13	1.01	1.25
-0.86	0.21	0.18	0.24	1.00	0.86	0.88
-1.34	0.22	0.21	0.20	0.95	1.12	0.93
<u>log conc.</u>	<u>NR2A - EC₃₀ glutamate</u>			<u>NR2A - EC₁₀₀ CGP070667</u>		
<u>compound</u>						
2.00	0.37	0.46	0.47	0.95	0.81	0.83
1.52	0.49	0.53	0.58	0.74	0.86	0.81
1.05	0.38	0.46	0.49	0.88	0.83	0.88
0.57	0.22	0.27	0.25	0.81	0.93	0.78
0.09	0.20	0.19	0.25	0.73	0.90	0.73
-0.39	0.10	0.08	0.07	0.84	0.73	0.80
-0.86	0.08	0.09	0.12	1.03	0.92	0.87
-1.34	0.10	0.12	0.15	1.00	0.96	1.03
<u>NR1/2B</u>						
<u>NR2B - EC₃₀ D-serine</u>			<u>NR2B - EC₁₀₀ MDL105,519</u>			
2.00	0.28	0.33	0.53	0.98	1.04	0.95
1.52	1.33	1.46	1.17	1.63	1.70	1.69
1.05	0.95	1.02	1.04	1.81	1.43	1.52
0.57	0.56	0.66	0.59	1.68	1.33	1.58
0.09	0.46	0.58	0.43	2.16	1.93	2.02
-0.39	0.33	0.20	0.21	1.95	1.48	1.44
-0.86	0.21	0.24	0.08	1.13	1.19	1.00
-1.34	0.28	0.33	0.20	0.99	1.01	1.00

TABLE 17
Effect of NMDAR Positive Allosteric Modulators
on NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux

	<u>NR2B - EC₃₀ glutamate</u>			<u>NR2B - EC₁₀₀ CGP070667</u>		
2.00	0.37	0.44	0.51	0.87	0.92	0.97
1.52	0.78	0.96	1.01	1.29	1.08	1.23
1.05	0.63	0.65	0.61	1.23	1.37	1.15
0.57	0.51	0.37	0.47	1.09	1.20	1.07
0.09	0.26	0.32	0.25	0.88	0.88	1.04
-0.39	0.14	0.20	0.23	1.14	0.83	0.87
-0.86	0.07	0.06	0.13	0.87	0.97	0.93
-1.34	0.17	0.12	0.30	0.89	1.12	0.99
	<u>1st interval</u>					
	<u>NR2A - MDL105,519</u>			<u>NR2A - CGP070667</u>		
2.00	1.00	1.04	1.03	1.19	1.18	1.23
1.52	1.08	1.08	1.06	1.22	1.22	1.23
1.05	1.07	1.00	1.01	1.14	1.15	1.14
0.57	0.99	0.99	0.99	1.08	1.08	1.08
0.09	0.95	0.97	0.92	1.03	1.00	1.00
-0.39	0.95	0.97	0.97	1.04	1.02	0.98
-0.86	0.96	0.95	0.97	0.99	0.98	0.97
-1.34	0.95	0.96	0.94	1.02	1.00	1.00
	<u>NR2B - MDL105,519</u>			<u>NR2B - CGP070667</u>		
2.00	1.01	1.03	1.03	1.16	1.18	1.21
1.52	1.16	1.18	1.13	1.37	1.42	1.43
1.05	1.12	1.10	1.09	1.25	1.27	1.24
0.57	1.03	1.03	1.02	1.17	1.13	1.15
0.09	1.01	1.01	0.99	1.07	1.08	1.06
-0.39	0.98	0.97	0.97	1.03	1.04	1.02
-0.86	0.97	0.97	0.96	1.02	1.01	1.01
-1.34	0.97	0.98	0.96	1.03	1.02	1.05
	<u>CIQ</u>					
	<u>NR1/2A</u>					
<u>log conc.</u>	<u>NR2A - EC₃₀ D-Serine</u>			<u>NR2A - EC₁₀₀ MDL105,519</u>		
<u>compound</u>						
2.00	0.21	0.16	0.18	0.60	0.67	0.66
1.52	0.20	0.17	0.18	0.75	0.63	0.67
1.05	0.18	0.17	0.19	0.58	0.84	0.88
0.57	0.19	0.15	0.16	0.82	0.94	0.83
0.09	0.11	0.19	0.14	0.80	0.80	0.83
-0.39	0.18	0.19	0.21	0.69	1.06	0.95
-0.86	0.21	0.16	0.22	1.08	0.81	0.92
-1.34	0.18	0.21	0.22	0.96	1.09	0.96
	<u>NR2A - EC₃₀ glutamate</u>			<u>NR2A - EC₁₀₀ CGP070667</u>		
2.00	0.16	0.15	0.15	0.42	0.45	0.53
1.52	0.07	0.04	0.07	0.72	0.51	0.56
1.05	0.06	0.03	0.14	0.60	0.58	0.76
0.57	0.04	0.03	0.09	0.66	0.71	0.76
0.09	0.09	0.16	0.20	0.64	0.62	0.75
-0.39	0.08	0.10	0.19	0.75	0.77	0.64
-0.86	0.14	0.11	0.20	1.00	0.80	0.83
-1.34	0.07	0.16	0.18	0.93	1.06	1.01

TABLE 17
Effect of NMDAR Positive Allosteric Modulators
on NR1/2A-Mediated or NR1/2B-Mediated Calcium Flux

<u>log conc.</u> <u>compound</u>	<u>NR1/2B</u>					
	<u>NR2B - EC₃₀ D-serine</u>			<u>NR2B - EC₁₀₀ MDL105,519</u>		
2.00	-0.16	-0.30	-0.17	-0.18	-0.21	0.03
1.52	-0.09	0.07	-0.11	0.05	0.18	0.10
1.05	0.05	0.00	-0.06	0.24	0.26	0.37
0.57	-0.04	0.11	-0.03	0.62	0.32	0.46
0.09	-0.04	0.18	0.12	0.93	1.20	1.06
-0.39	0.23	0.18	0.18	1.05	1.01	0.94
-0.86	0.20	-0.01	0.06	0.75	0.66	0.64
-1.34	0.18	0.42	0.27	1.05	1.01	0.94
<u>log conc.</u> <u>compound</u>	<u>NR2B - EC₃₀ glutamate</u>			<u>NR2B - EC₁₀₀ CGP070667</u>		
2.00	0.07	0.10	0.04	0.58	0.70	0.50
1.52	0.06	0.01	0.03	0.68	0.51	0.73
1.05	0.00	0.08	0.06	0.80	0.76	0.76
0.57	0.05	0.11	0.10	1.11	0.93	0.81
0.09	0.11	0.07	0.20	0.83	0.87	0.83
-0.39	0.29	-0.01	0.21	0.95	0.91	0.79
-0.86	0.06	0.01	0.17	1.13	1.09	0.89
-1.34	0.19	0.25	0.23	0.93	1.03	1.03
<u>log conc.</u> <u>compound</u>	<u>1st interval</u>					
	<u>NR2A - MDL105,519</u>			<u>NR2A - CGP070667</u>		
2.00	0.88	0.85	0.85	0.96	0.96	0.94
1.52	0.89	0.90	0.89	0.95	0.94	0.96
1.05	0.91	0.92	0.92	0.95	0.95	0.96
0.57	0.94	0.91	0.91	0.95	0.94	0.95
0.09	0.90	0.91	0.88	0.97	0.96	0.98
-0.39	0.95	0.92	0.95	0.97	0.97	1.00
-0.86	0.97	0.93	0.96	0.99	0.96	1.00
-1.34	0.93	0.93	0.94	0.98	1.01	1.01
<u>log conc.</u> <u>compound</u>	<u>NR2B - MDL105,519</u>			<u>NR2B - CGP070667</u>		
2.00	0.89	0.86	0.88	0.94	0.95	0.92
1.52	0.91	0.93	0.92	0.94	0.93	0.94
1.05	0.95	0.95	0.93	0.94	0.98	0.96
0.57	0.93	0.95	0.94	0.96	0.98	0.97
0.09	0.94	0.97	0.96	0.99	0.98	1.01
-0.39	0.98	0.97	0.97	1.03	0.99	1.00
-0.86	0.97	0.95	0.96	1.00	1.00	1.01
-1.34	0.97	0.98	0.96	1.01	1.05	1.02

Data and time intervals are rounded to two decimal places

- [0137]** Spermine, an extracellular polyamine, has been described to enhance NMDA receptor responses by both ligand-dependent and ligand-independent mechanisms. Addition of Spermine in our assay in the presence of submaximal concentration of D-Serine and saturating concentration of glutamate enhanced NMDAR activity in both NR1/2A and NR1/2B expressing cells. See, TABLE 17. Spermine had little effect in the presence of submaximal concentrations of glutamate and had no effect in the absence of exogenously added ligand. These data indicate a D-serine-dependent effect of spermine on both NMDAR subtypes, likely by increasing the sensitivity to D-serine, as previously found in electrophysiological studies. In the presence of saturating amounts of ligand,

spermine enhanced the activity of NR1/2B heterodimers, but not of NR1/2A heterodimers, demonstrating the known D-serine-independent and NR1/2B specific effect of polyamines on NMDAR activity. Interestingly, spermine did not potentiate the activity of NR1/2B under saturating concentrations of ligand when cells were protected with the glutamate binding site antagonist. This observation suggests that spermine has a stronger effect when incubated with NMDAR at low D-serine concentrations (in MDL105,519-protected cells), followed by addition of saturating amounts of D-serine as compared to addition of spermine in the presence of high concentration of D-serine (in CGP070667-protected cells). Similar effects were described in the scientific literature for other positive allosteric modulators.

[0138] As a second example, we tested pregnenolone sulfate (PS), a naturally occurring compound that causes potentiation of NMDAR by increasing channel open probability. Horak M *et al.*, *J Neurosci.* 24(46): 10318-25 (17 November 2004). In our assay of the invention, pregnenolone sulfate enhanced NMDAR activity in the presence of submaximal concentrations of D-serine and glutamate in both NR1/2A and NR1/2B containing heterodimers, with a slightly higher potency in NR1/2B. See, TABLE 17. Comparable activation was observed in the presence of limited concentration of either ligand, suggesting a ligand-independent mode of action of pregnenolone sulfate on NMDAR. Pregnenolone sulfate caused supermaximal activation of NMDAR in the presence of saturating concentrations of ligand and furthermore caused a weak activation of NMDAR in the absence of exogenously added ligand, further supporting the mode of a ligand-independent mechanism of action and being in line with the proposed mechanism of action of pregnenolone sulfate to increase channel open probability. These data demonstrate the ability of our assay of the invention to identify positive allosteric modulators with ligand-dependent and ligand-independent modes of action.

[0139] As a third example, we tested CIQ, a known NR2C and NR2D selective positive allosteric modulator. In the assay of the invention, CIQ had no potentiating effects on NMDAR in any tested condition, demonstrating the specificity of our assay for NR1/2A and NR1/2B PAMs. See, TABLE 17.

[0140] Lastly, we set out to characterize the effect of the NR2A-specific positive allosteric modulator GNE-6901, published in WO 2015/052226 (Genentech, Inc.). In our assay, GNE-6901 caused a concentration-dependent potentiation of NR1/2A activity at submaximal concentrations of D-serine or glutamate. See, TABLE 17. At the highest concentration tested, GNE-6901 potentiated NR1/2A activity in the presence of EC₃₀ of either ligand to the same level as observed in the presence of saturating concentrations of both ligands. At saturating concentrations of ligand, GNE-6901 potentiated NMDAR activity even further, resulting in a super maximal activation of NR1/2A..

[0141] In further support of this determination, we found GNE-6901 caused a significant activation of NR1/2A in the absence of exogenously added ligand. This activity was strongest in cells protected with CGP070667, indicating a ligand-dependent mode of action for GNE-6901, by increasing the sensitivity to glutamate. GNE-6901 had no effect on NR1/2B, highlighting the reported specificity of the compound for NR1/2A.

[0142] . Taken together, the data presented here introduces a novel dual-ligand sensitive model system for studying NMDAR biology and modulation, including the characterization of NMDAR inhibitors as well as positive allosteric modulators. Thus, the assay of the invention can be used to enable the identification of novel therapeutics for various NMDAR-dependent pathologies.

EXAMPLE X

Comparison of the assay of published patent application WO 2015/052226 (Genentech) with the assay of the invention

[0143] We compared the assay conditions of a patent application published in 2015 with the assay conditions in the assay of the invention. The comparison is presented in TABLE 18.

TABLE 18		
	<u>Assay of WO 2015/052226 A1</u>	<u>Assay of the invention</u>
Cell line	HEK-tet-hNR1/2A, induction over night	HEKwt BacMam hNR1/2A, infection over night
Cultured in DMEM	+ 500 μ M ketamine	+ 100 μ M MDL105.519 or 3 μ M CGP070667
Assay Kit	BD Calcium assay	Calcium 6 dye or GCaMP variants
Assay buffer	HBSS with 1.8 mM CaCl_2 , no Mg^{2+} ; 200 μ M probenecid, 10 μ M ketamine	HBSS with 1.8 mM CaCl_2 , no Mg^{2+} ; with calcium 6 dye
Ligand	30 μ M glycine + 300 nM L-glutamate (EC_{30})	EC_{100} 100 μ M glycine/L-glutamate EC_{30} 5 μ M glycine/ L-glutamate
Sensitivity	L-glutamate only	glycine or L-glutamate
Order of addition	Ligand + compounds added together, 5 minutes measurement	Compound first, 5 minutes; Ligand second, 5 minutes
Readout	Max ratio	Integral of max ratio
Data representation	% EC_{100} stimulated control	Fold DMSO control

20 EXAMPLE XI

Utility of calcium sensor for high throughput screening

[0144] GCaMP is a genetically encoded calcium indicator, or GECI. The calcium sensor reagent for this EXAMPLE (dCys-GCaMP6) is a particular mutant of a known GCaMP calcium sensor. We have developed an assay using our calcium sensor in iPSCs in a high-throughput format. This assay can readily be adapted for other iPSCs of interest.

EXAMPLE XIIUtility of the assay of the invention to test ligand stimulation of NMDAR (NR1/NR2D)

- [0145]** We transduced HEK293 cells with NR1/2A and protected the cells with
- 5 MDL105,519 or CGP070667, using the method described above in EXAMPLE VI and EXAMPLE VII.

TABLE 18
Sensitivity of NMDAR (NR1/NR2D) mediated calcium flux to ligand stimulation

concentration ligand in μM	100 μM D-serine, L-glutamate titration			
300.00	1.52	1.45	1.52	1.40
150.00	1.36	1.47	1.34	1.32
75.00	1.47	1.40	1.37	1.34
37.50	1.26	1.22	1.41	1.28
18.75	1.27	1.23	1.22	1.24
9.38	1.14	1.11	1.22	1.16
4.69	1.08	1.02	1.13	1.11
2.34	1.05	1.00	1.07	0.99
1.17	1.03	1.03	1.06	0.99
0.59	1.11	1.01	1.04	0.97
0.29	1.08	1.02	1.02	1.00
0.15	1.00	1.00	1.00	1.00
	100 μM L-glutamate, D-serine titration			
300.00	1.46	1.49	1.39	1.53
150.00	1.41	1.52	1.36	1.32
75.00	1.38	1.31	1.33	1.39
37.50	1.32	1.31	1.39	1.38
18.75	1.28	1.26	1.25	1.41
9.38	1.27	1.21	1.22	1.26
4.69	1.32	1.28	1.17	1.17
2.34	1.18	1.11	1.05	1.11
1.17	1.04	1.04	1.01	1.02
0.59	1.07	1.01	1.07	1.00
0.29	1.05	1.06	1.01	1.02
0.15	1.00	1.00	1.00	1.00

10 EMBODIMENTS

[0146] The invention provides the following embodiments:

[0147] 1. A method for identifying a modulator of N-methyl-D-aspartate receptor (NMDAR) function, comprising the steps of:

- (a) transiently expressing an NMDAR protein or subunit thereof on the surface of a
- 15 mammalian cell;
- (b) culturing the mammalian cell in a culture medium, where the culture medium:
- (i) contains an NMDAR ligand binding site antagonist; and
 - (ii) does not contain an NMDAR channel blocker;

- (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
 - (d) while retaining the mammalian cell, replacing (i) the culture medium that contains the NMDAR ligand binding site antagonist and does not contain an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker;
 - (e) measuring the calcium response by the mammalian cell;
 - (f) contacting the mammalian cell with a test agent;
 - (g) measuring the calcium response by the mammalian cell to the test agent;
 - (h) contacting the mammalian cell with an NMDAR ligand site binder; and
 - (i) measuring the calcium response by the mammalian cell;
- wherein a change in calcium response by the mammalian cell identifies the test agent as a modulator of NMDAR function.
- [0148]** 2. The method of embodiment 1, wherein transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector comprising one or more NMDAR genes, and expression of the NMDAR genes on the vector.
- [0149]** 3. The method of embodiment 2, wherein the vector is a baculovirus vector comprising one or more NMDAR genes.
- [0150]** 4. The method of embodiment 1, wherein transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a stable transfection of the mammalian cell with one or more vectors comprising one or more NMDAR genes, wherein one or more of the NMDAR genes is inducible.
- [0151]** 5. The method of embodiment 1, wherein the NMDAR is selected from the group consisting of NR1/2A, NR1/2B and NR1/2D.
- [0152]** 6. The method of embodiment 1, wherein the NMDAR subunit is selected from the group consisting of NR1, NR2A, NR2B, and NR2D.
- [0153]** 7. The method of embodiment 1, wherein the NMDAR is a human or mouse NMDAR.
- [0154]** 8. The method of embodiment 1, wherein the mammalian cell is a human cell, such a HEK293 or a human iPSC.
- [0155]** 9. The method of embodiment 1, wherein the NMDAR ligand binding site antagonist binds to the glycine binding site.
- [0156]** 10. The method of embodiment 1, wherein the NMDAR ligand binding site antagonist binds to the glutamate binding site.
- [0157]** 11. The method of embodiment 1, wherein the NMDAR ligand binding site antagonist is selected from the group consisting of MDL105,519 and CGP070667 .

- [0158] 12. The method of embodiment 1, wherein manipulating the mammalian cell so that it responds in a measurable way to changes in calcium concentration in the mammalian cell is by loading the mammalian cell with a cell-permeable calcium indicator.
- [0159] 13. The method of embodiment 16, wherein in the calcium indicator is calcium 6 dye.
- [0160] 14. The method of embodiment 1, wherein the manipulating the mammalian cell so that it is responds in a measurable way to changes in calcium concentration in the mammalian cell is by stably expressing a calcium sensor protein in the mammalian cell.
- [0161] 15. The method of embodiment 14, wherein the calcium sensor protein is stably expressed in the mammalian cell by expression from a lentivirus vector comprising a calcium sensor protein gene.
- [0162] 16. The method of embodiment 1, wherein the measuring of the calcium response by the mammalian cell is in a high-throughput screen.
- [0163] 17. The method of embodiment 1, wherein the NMDAR ligand site binder binds to the NMDAR glycine binding site.
- [0164] 18. The method of embodiment 1, wherein the NMDAR ligand site binder binds to the NMDAR glutamate binding site.
- [0165] 19. The method of embodiment 1, wherein the modulator of NMDAR function is an NMDAR positive allosteric modulator, as detected by an increase in calcium response.
- [0166] 20. The method of embodiment 1, wherein the modulator of NMDAR function is an NMDAR negative inhibitor, as detected by a decrease in calcium response.
- [0167] 21. A mammalian cell having a high level of an N-methyl-D-aspartate receptor (NMDAR) protein on the cell surface, wherein:
- (a) the mammalian cell comprises a vector containing an expressible NMDAR gene, wherein:
 - (i) the vector is a baculovirus vector;
 - (ii) a vector for transient transfection; or
 - (iii) the vector is integrated in the mammalian cell genome, and measurable by resistance to a selection marker encoded by the vector;
 - (c) the expressible NMDAR gene is expressed in the mammalian cell;
 - (d) the expressed NMDAR protein or subunit thereof is present on the cell surface; and
 - (e) the mammalian cell is viable.
- [0168] 22. The mammalian cell of embodiment 21, wherein the transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient

transfection of the mammalian cell with a vector one or more NMDAR genes and expression of the NMDAR genes on the vector.

[0169] 23. The mammalian cell of embodiment 22, wherein the vector is a baculovirus vector comprising one or more NMDAR genes.

5 **[0170]** 24. A method for producing a viable mammalian cell having a high level of N-methyl-D-aspartate receptor (NMDAR) on its surface, comprising the steps of:

- (a) transiently transfecting the mammalian cell with a vector selected from the group consisting of:
 - (i) baculovirus vector containing an expressible NMDAR gene, and
 - 10 (ii) a plasmid coding for NMDAR; and
- (b) culturing the mammalian cell, where the culture medium
 - (i) contains an NMDAR ligand binding site antagonist; and
 - (ii) does not contain an NMDAR channel blocker.

15 **[0171]** 25. The method of embodiment 24, wherein the transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector comprising one or more NMDAR genes and expression of the NMDAR genes on the vector.

[0172] 26. The method of embodiment 25, wherein the vector is a baculovirus vector comprising one or more NMDAR genes.

20 **[0173]** 27. A method for facilitating the sensitivity of an N-methyl-D-aspartate receptor (NMDAR) to ligands of the NMDAR glycine site or to the NMDAR glutamate site, comprising the steps of:

- (a) transiently expressing NMDAR protein or subunits thereof on the surface of a mammalian cell;
- 25 (b) culturing the mammalian cell in a culture medium, wherein the culture medium
 - (i) contains an NMDAR ligand binding site antagonist; and
 - (ii) does not contain an NMDAR channel blocker;
- (c) while retaining the mammalian cell, replacing (i) the culture medium that contains the NMDAR ligand binding antagonist and does not contain an NMDAR channel
- 30 blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker;
- (d) contacting the mammalian cell with a test agent;
- (e) contacting the mammalian cell with an NMDAR ligand site binder; and
- (f) determining whether the test agent binds to the glycine binding site or the
- 35 glutamate binding site.

[0174] 28. The method of embodiment 27, wherein the transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient

transfection of the mammalian cell with a vector one or more NMDAR genes and expression of the NMDAR genes on the vector.

[0175] 29. The method of embodiment 28, wherein the vector is a baculovirus vector comprising one or more NMDAR genes.

5 **[0176]** 30. A method for dissecting the mechanism of action of N-methyl-D-aspartate receptor (NMDAR) inhibitors, comprising the steps of:

- (a) transiently expressing NMDAR protein or subunits thereof on the surface of a mammalian cell;
- (b) culturing the mammalian cell, where the culture medium
 - 10 (i) contains an NMDAR ligand binding antagonist; and
 - (ii) does not contain an NMDAR channel blocker;
- (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
- (d) while retaining the mammalian cell, replacing (i) the culture medium that contains
 - 15 the NMDAR ligand binding antagonist and does not contain an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker;
- (e) measuring the calcium response by the mammalian cell;
- (f) contacting the mammalian cell with a test agent;
- 20 (g) measuring the calcium response by the mammalian cell to the test agent;
- (h) contacting the mammalian cell with an NMDAR ligand site binder;
- (i) measuring the calcium response by the mammalian cell;
- (j) determining whether the test agent binds to the glycine binding site or the glutamate binding site.

25 **[0177]** 31. The method of embodiment 30, wherein the transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector comprising one or more NMDAR genes and expression of the NMDAR genes on the vector.

[0178] 32. The method of embodiment 31, wherein the vector is a baculovirus vector
 30 comprising one or more NMDAR genes.

[0179] 33. A method for detecting calcium levels in a eukaryotic cell, comprising the steps of:

- (a) transfecting the eukaryotic cell with a nucleotide comprising an expressible gene encoding a genetically encoded calcium sensor (GECS)-based GCaMP-protein;
- (b) expressing the gene for a genetically encoded calcium sensor to create a eukaryotic cell stably expressing GCaMP protein;
- 5 (c) contacting the eukaryotic cell with a test agent; and
- (d) measuring the calcium response by the eukaryotic cell to the test agent;
- [0180]** 34. The method of embodiment 33, wherein the nucleotide is a lentivirus.
- [0181]** 35. The method of embodiment 34, wherein the expressible gene encodes GCaMP6.
- 10 **[0182]** 36. A method of screening for a positive allosteric modulator (PAM) of N-methyl-D-aspartate receptor (NMDAR) for use in treating or palliating schizophrenia, comprising the steps of:
 - (a) transiently expressing NMDAR protein or NR1 or NR2 subunits thereof on the surface of a mammalian cell;
 - 15 (b) culturing the mammalian cell, where the culture medium:
 - (i) contains an NMDAR ligand binding antagonist; and
 - (ii) does not contain an NMDAR channel blocker;
 - (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
 - 20 (d) measuring the calcium response by the mammalian cell;
 - (e) contacting the mammalian cell with a test agent;
 - (f) measuring the calcium response by the mammalian cell to the test agent;
 - (g) contacting the mammalian cell with an NMDA ligand site binder; and
 - (h) measuring the calcium response by the mammalian cell;
- 25 wherein an increase in calcium response by the mammalian cell identifies the test agent as a positive allosteric modulator of an NMDAR for use in treating schizophrenia.
- [0183]** 37. The method of embodiment 36, wherein the transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector comprising one or more NMDAR genes and expression of the NMDAR genes on the vector.
- 30 **[0184]** 38. The method of embodiment 37, wherein the vector is a baculovirus vector comprising one or more NMDAR genes.
- [0185]** The contents of each of the patents and publications cited herein are
- 35 incorporated by reference in their entirety.

[0186] The detailed description provided herein is to illustrate the invention, but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the biotechnological arts and are encompassed by the appended claims.

SEQUENCE LISTING

<u>SEQ ID NOs. and Sequences</u>	
SEQ ID NO: 1	dCys-GCAMP6s = GCAMP6s T330C/T364C
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SEQ ID NO: 2	CGaMP3 WO 2011/056975 A9 (Howard Hughes Medical Institute)
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SEQ ID NO: 5	pLVX-Puro_dCys-GAMP6s for expression of dCys-GAMP6s
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SEQ ID NO: 6

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CLAIMS

1. A method for identifying a modulator of N-methyl-D-aspartate receptor (NMDAR) function, comprising the steps of:
- 5 (a) transiently expressing an NMDAR protein or subunit thereof on the surface of a mammalian cell;
- (b) culturing the mammalian cell in a culture medium, where the culture medium:
- 10 (i) contains an NMDAR ligand binding site antagonist; and
- (ii) does not contain an NMDAR channel blocker;
- (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
- (d) while retaining the mammalian cell, replacing (i) the culture medium that contains the NMDAR ligand binding site antagonist and does not contain
- 15 an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker;
- (e) measuring the calcium response by the mammalian cell;
- (f) contacting the mammalian cell with a test agent;
- (g) measuring the calcium response by the mammalian cell to the test agent;
- 20 (h) contacting the mammalian cell with an NMDAR ligand site binder; and
- (i) measuring the calcium response by the mammalian cell;
- wherein a change in calcium response by the mammalian cell identifies the test agent as a modulator of NMDAR function.
- 25 2. The method of claim 1, wherein transiently expressing NMDAR protein or subunit thereof on the surface of the mammalian cell is by a transient transfection of the mammalian cell with a vector comprising one or more NMDAR genes, and expression of the NMDAR genes on the vector.
- 30 3. The method of claim 1, wherein the NMDAR is selected from the group consisting of NR1/2A, NR1/2B and NR1/2D.
4. The method of claim 1, wherein manipulating the mammalian cell so that it responds in a measurable way to changes in calcium concentration in the
- 35 mammalian cell is by loading the mammalian cell with a cell-permeable calcium indicator.

5. The method of claim 1, wherein the manipulating the mammalian cell so that it is responds in a measurable way to changes in calcium concentration in the mammalian cell is by stably expressing a calcium sensor protein in the mammalian cell.
- 5
6. The method of claim 1, wherein the measuring of the calcium response by the mammalian cell is in a high-throughput screen.
7. The method of claim 1, wherein the modulator of NMDAR function is an NMDAR positive allosteric modulator, as detected by an increase in calcium response.
- 10
8. The method of claim 1, wherein the modulator of NMDAR function is an NMDAR negative inhibitor, as detected by a decrease in calcium response.
- 15
9. A mammalian cell having a high level of an N-methyl-D-aspartate receptor (NMDAR) protein on the cell surface, wherein:
- (a) the mammalian cell comprises a vector containing an expressible NMDAR gene, wherein:
- (i) the vector is a baculovirus vector;
- (ii) a vector for transient transfection; or
- (iii) the vector is integrated in the mammalian cell genome, and measurable by resistance to a selection marker encoded by the vector;
- (c) the expressible NMDAR gene is expressed in the mammalian cell;
- (d) the expressed NMDAR protein or subunit thereof is present on the cell surface; and
- (e) the mammalian cell is viable.
- 20
- 25

10. A method for dissecting the mechanism of action of N-methyl-D-aspartate receptor (NMDAR) inhibitors, comprising the steps of:
- (a) transiently expressing NMDAR protein or subunits thereof on the surface of a mammalian cell;
 - 5 (b) culturing the mammalian cell, where the culture medium
 - (i) contains an NMDAR ligand binding antagonist; and
 - (ii) does not contain an NMDAR channel blocker;
 - (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
 - 10 (d) while retaining the mammalian cell, replacing (i) the culture medium that contains the NMDAR ligand binding antagonist and does not contain an NMDAR channel blocker with (ii) a buffer or culture medium that does not contain an NMDAR channel blocker;
 - (e) measuring the calcium response by the mammalian cell;
 - 15 (f) contacting the mammalian cell with a test agent;
 - (g) measuring the calcium response by the mammalian cell to the test agent;
 - (h) contacting the mammalian cell with an NMDAR ligand site binder;
 - (i) measuring the calcium response by the mammalian cell;
 - 20 (j) determining whether the test agent binds to the glycine binding site or the glutamate binding site.
11. A method for detecting calcium levels in a eukaryotic cell, comprising the steps of:
- (a) transfecting the eukaryotic cell with a nucleotide comprising an
25 expressible gene encoding a genetically encoded calcium sensor (GECS)-based GCaMP-protein;
 - (b) expressing the gene for a genetically encoded calcium sensor to create a eukaryotic cell stably expressing GCaMP protein;
 - (c) contacting the eukaryotic cell with a test agent; and
 - 30 (d) measuring the calcium response by the eukaryotic cell to the test agent;
12. The method of claim 36, wherein the expressible gene encodes dCys-GCAMP6 (SEQ ID NO: 1); GCAMP3 (SEQ ID NO: 2) or GCAMP6 (SEQ ID NO: 3).

13. A method of screening for a positive allosteric modulator (PAM) of N-methyl-D-aspartate receptor (NMDAR) for use in treating or palliating schizophrenia, comprising the steps of:
- 5 (a) transiently expressing NMDAR protein or NR1 or NR2 subunits thereof on the surface of a mammalian cell;
 - (b) culturing the mammalian cell, where the culture medium:
 - (i) contains an NMDAR ligand binding antagonist; and
 - (ii) does not contain an NMDAR channel blocker;
 - 10 (c) manipulating the mammalian cell to respond in a measurable way to changes in calcium concentration in the mammalian cell;
 - (d) measuring the calcium response by the mammalian cell;
 - (e) contacting the mammalian cell with a test agent;
 - (f) measuring the calcium response by the mammalian cell to the test agent;
 - (g) contacting the mammalian cell with an NMDA ligand site binder; and
 - 15 (h) measuring the calcium response by the mammalian cell;
- wherein an increase in calcium response by the mammalian cell identifies the test agent as a positive allosteric modulator of an NMDAR for use in treating schizophrenia.

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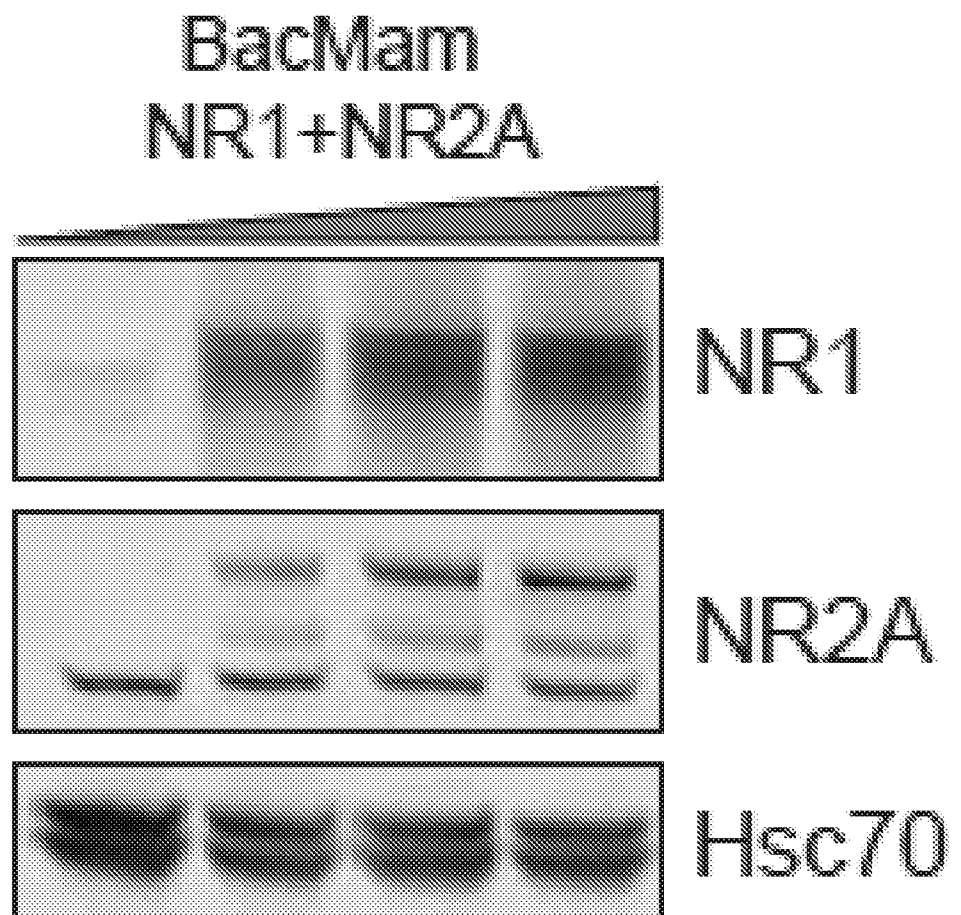


FIG. 1

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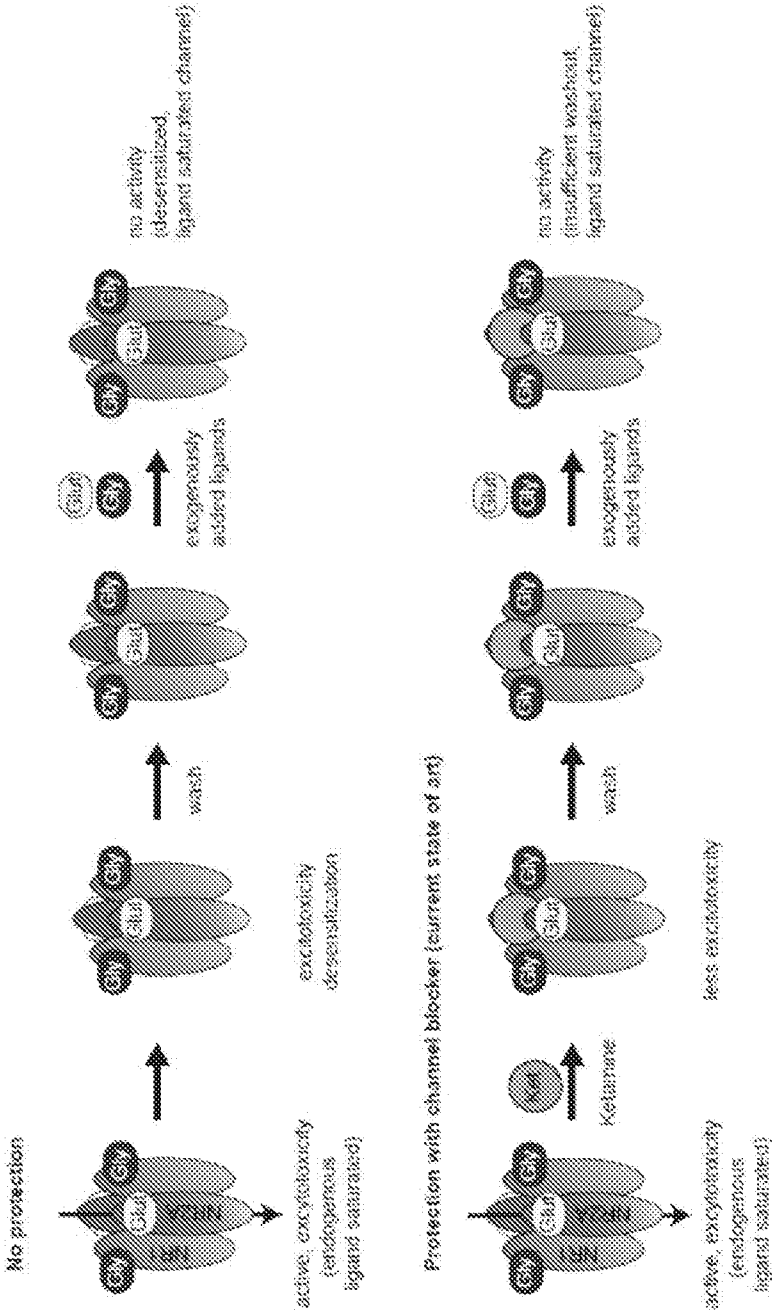


FIG. 2

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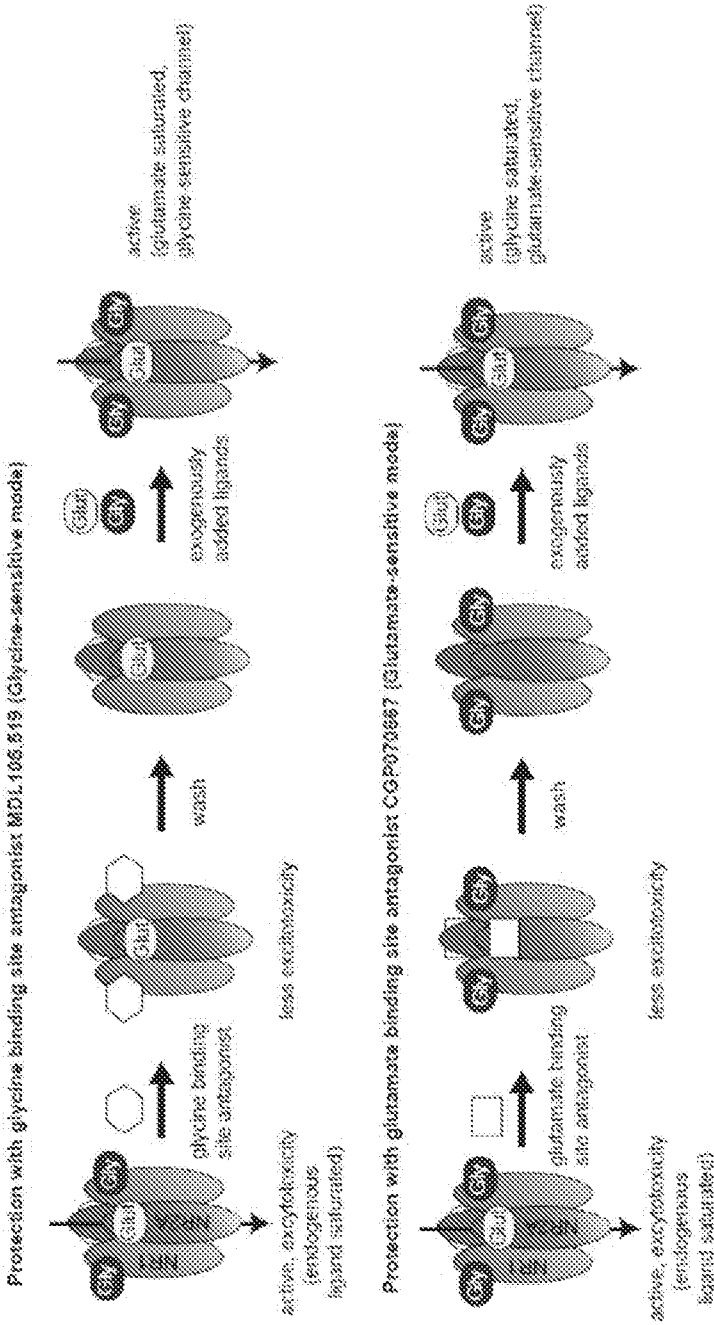


FIG. 3

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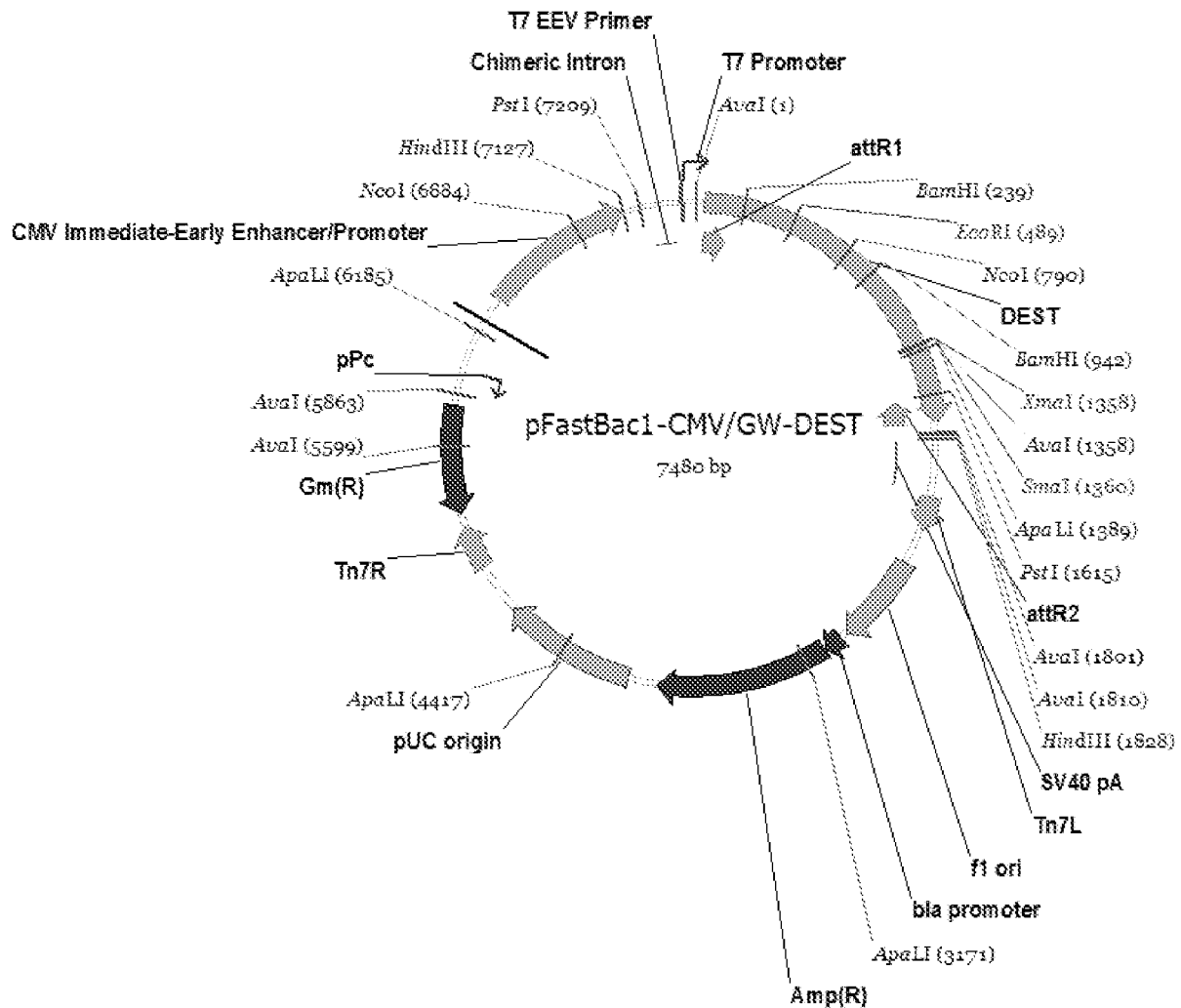


FIG. 4