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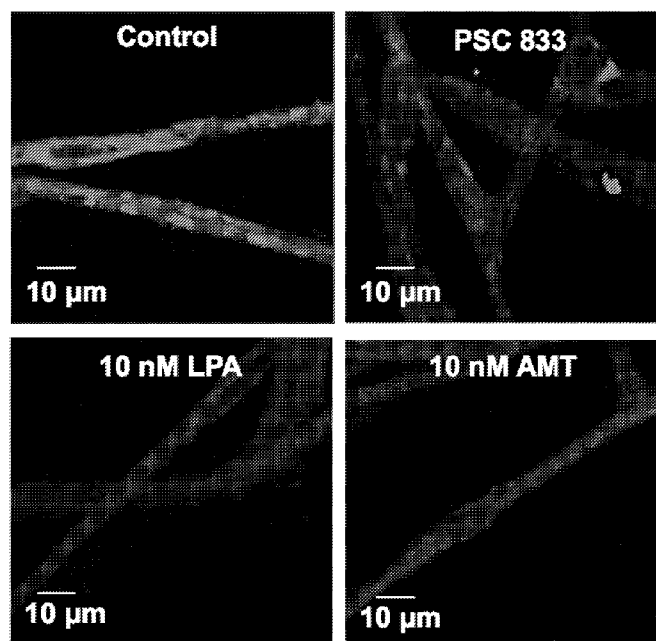


Figure 12

(57) **Abstract:** Described herein are novel methods and compositions useful in modulating the permeability of a P-glycoprotein expressing barrier of a tissue or an organ, such as the blood- brain barrier, of a subject and improving transport of an agent across the barrier by co- administration of the agent with a LPAI Receptor ligand, wherein the LPAI Receptor ligand is administered at a concentration sufficient to reversibly inhibit transport activity of P glycoprotein. Further described herein are compositions and methods useful for treating a condition or disease in a subject by administering to the subject a composition that comprises a therapeutic agent effective against the condition or disease and an LPAI Receptor ligand at a concentration sufficient to reversibly inhibit transport activity of P glycoprotein thereby increasing transport of the therapeutic agent across a P-glycoprotein expressing barrier of a tissue or an organ, such as the blood brain barrier of the subject. In some embodiments, the LPAI Receptor ligand may be an antidepressant, including



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DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR,
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MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
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METHODS FOR IMPROVING DRUG DELIVERY ACROSS A P-GLYCOPROTEIN EXPRESSING BARRIER

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application Serial No. 62/332,888, filed May 6, 2016, and U.S. Provisional Application Serial No. 62/453,718, filed February 2, 2017, all of which are incorporated herein by reference in their entireties for all purposes.

GOVERNMENT SUPPORT

10 The government has certain rights in this invention.

TECHNICAL FIELD

 The present invention relates to methods and compositions useful in modulating the permeability of the blood-brain barrier and to improvements in the field of drug delivery. More particularly, the invention relates to a reversible method for enhancing the transport
15 of a molecule across the blood-brain barrier of an individual.

BACKGROUND

 The blood-brain barrier (BBB) is a dynamic interface that separates the brain from the circulatory system and protects the central nervous system (CNS) from potentially harmful chemicals while regulating transport of essential molecules and maintaining a stable
20 environment. The blood-brain barrier (BBB) is considered as a major obstacle for the potential use of drugs for treating disorders of the central nervous system (CNS). More than 98% of all potential CNS drugs do not cross the blood-brain barrier. In addition, more than 99% of worldwide CNS drug development is devoted solely to CNS drug discovery, and less than 1% is directed to CNS drug delivery. This ratio could justify why no efficient

treatment is currently available for the major neurological diseases such as brain tumors, Alzheimer's disease and stroke.

The selective permeability of the blood-brain barrier is maintained by a combination of tight junctions between brain capillary endothelial cells and selective ATP-binding cassette (ABC) transporters that guide the directed transport of both endogenous and exogenous agents between brain parenchyma and systemic circulation. The transport function of these membrane proteins has two major consequences: neuroprotection from environmental toxins and limited drug delivery to the central nervous system (CNS). However, therapeutics developed to treat diseases of the central nervous system (CNS) must cross this interface in order to reach full clinical potential.

P-glycoprotein is an ABC transporter protein and a major player at this dynamic interface for several reasons: high expression relative to other ABC transporters, an extensive list of clinically relevant substrates, and the potential to modulate its activity via manipulation of the intracellular signaling processes that regulate it. P-glycoprotein causes a net efflux of its substrates from the brain and blocks entry of drugs into the brain. Thus, it is a major obstacle for the targeted delivery of many CNS-targeted drugs, for example chemotherapeutics, pain management associated drugs and the Amyotrophic Lateral Sclerosis (ALS) drug riluzole.

Its high expression relative to the other transporters at the blood-brain interface and a directionality that results in the net efflux of its clinical substrates from brain to blood directly oppose efforts made by physicians to treat their patients with drugs whose direct targets lie just beyond the brain microvasculature. For CNS diseases like epilepsy and ALS, bypassing P-glycoprotein-driven pharmacoresistance is especially challenging because their development either involves or drives P-glycoprotein upregulation directly.

P-glycoprotein's activity is the primary reason that CNS drugs fail to make it to the market, as it hinders 95% of all CNS chemotherapeutics and 70% of the most widely prescribed drugs. Efforts to transiently decrease the transport activity of P-glycoprotein in order to enhance the limited delivery of its substrates have been plentiful within the last decade. One such effort focused on the ability of the endogenous phospholipid sphingosine-1-phosphate (S1P) to signal through a discrete cascade at the capillary endothelium in order to achieve this reduction. However, the doses and mode of administration required oppose immediate clinical implementation.

Accordingly, there exists a need in the art for methods and compositions for facilitating and improving transport of therapeutic or diagnostic molecules across the blood-brain barrier by regulating the activity of P-glycoprotein. This application addresses such a need.

SUMMARY

In some embodiments, the present invention includes a method for delivery of an agent across a P-glycoprotein expressing barrier of a tissue or an organ of a subject comprising co-administering the agent with a LPA Receptor ligand, wherein the LPA Receptor ligand is administered in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.

In some embodiments, the present invention includes a method of treating, preventing or ameliorating a condition in a subject, comprising administering to a subject a composition, wherein the composition comprises a therapeutic agent effective against the condition and an LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein thereby increasing delivery of the therapeutic agent across a P-glycoprotein expressing barrier of a tissue or an organ of the subject.

In some embodiments, the present invention includes a method for modulating permeability of a P-glycoprotein expressing barrier of a tissue or an organ of a subject comprising administering to the subject a LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.

5 In some embodiments, the tissue or organ is selected from brain, kidney, liver, intestine, retina, placenta and testes. In some embodiments, the P-glycoprotein expressing barrier is blood brain barrier.

In some embodiments, the present invention includes a method for delivery of an agent across the blood brain barrier of a subject comprising co-administering the agent with
10 a LPA Receptor ligand, wherein the LPA Receptor ligand is administered in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.

In some embodiments, the present invention includes a method of treating, preventing or ameliorating a condition, comprising administering to a subject a composition, wherein the composition comprises a therapeutic agent effective against the condition and
15 an LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein thereby increasing delivery of the therapeutic agent across the blood brain barrier of the subject.

In some embodiments, the present invention includes a method for modulating permeability of the blood brain barrier of a subject comprising administering to the subject
20 a LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.

In some embodiments of the methods of the present invention, the integrity or physical permeability of the blood brain barrier is not affected. In some embodiments, the LPA Receptor ligand is a LPA1 Receptor ligand, a LPA3 Receptor ligand, or both. In some
25 embodiments, the LPA Receptor ligand is LPA or a structural analog of LPA. In some

embodiments, the LPA Receptor ligand is selected from the group consisting of LPA, NAEPA, OMPT, oleoyl-thiophosphate, 2-oleoyl-LPA, and T13. In some embodiments, the LPA Receptor ligand is LPA. In some embodiments, the LPA Receptor ligand is an antidepressant. In some embodiments, the antidepressant is selected from the group consisting of fluoxetine, amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine. In some embodiments, the LPA Receptor ligand is a tricyclic antidepressant. In some embodiments, the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine. In some embodiments, the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline and mianserin. In some embodiments, the LPA Receptor ligand is amitriptyline. In some embodiments, the LPA Receptor ligand is a selective ligand of LPA1 Receptor.

In some embodiments, the agent is a therapeutic agent or a diagnostic agent. In some embodiments, the agent is a therapeutic agent selected from the group consisting of an anti-bacterial agent, an anti-viral agent, an anti-mycotic agent, an anti-cancer agent, an analgesic, and a chemotherapeutic agent. In some embodiments, the agent is a therapeutic agent and is a drug molecule. In some embodiments, the agent is a diagnostic agent selected from the group consisting of an imaging molecule, and a labeling molecule.

In some embodiments, the agent and the LPA Receptor ligand are administered concurrently. In some embodiments, the agent and the LPA Receptor ligand are administered sequentially. In some embodiments, the route of administration of the agent or the LPA Receptor ligand or both is selected from the group consisting of intravenous, subcutaneous, transdermal, intramuscular, topical, and oral.

In some embodiments, delivery of the agent across the blood-brain barrier is increased about two-fold to about ten-fold compared to delivery of the agent in the absence of the LPA1 Receptor ligand. In some embodiments, the amount of the LPA Receptor ligand is sufficient to achieve a blood concentration level of about 0.1 to 1000.0 nM. In some
5 embodiments, the subject is a human.

In some embodiments, the condition is selected from the group consisting of a cancer, a neurological disease, and a pain. In some embodiments, the condition is cancer selected from the group consisting of brain cancer, neuroma, glioma, lymphoma, and glioblastoma. In some embodiments, the condition is neurological disease selected from the
10 group consisting of amyotrophic lateral sclerosis (ALS), brain tumor, brain metastasis, schizophrenia, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, a disease associated with malfunction of the BBB, dementia, and HIV-associated dementia. In some embodiments, the condition is pain selected from the group consisting of centrally mediated pain, peripherally mediated pain, neuropathic pain, acute pain, and
15 chronic pain.

In some embodiments, the present invention includes a pharmaceutical composition comprising a LPA Receptor ligand, an agent having therapeutic or diagnostic activity and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated as a solid dosage form. In some embodiments, the
20 pharmaceutical composition is formulated as a liquid dosage form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing depicting the blood brain barrier.

Figure 2 shows the chemical structure of lysophosphatidic acid (LPA) and a tricyclic antidepressant (TCA).

Figure 3 is a schematic drawing depicting the experimental design for conducting substrate transport assay, immunohistochemistry and Western blotting analysis on isolated brain capillaries.

Figure 4A-C shows that exposure to LPA reduces P-glycoprotein transport activity in a dose-dependent, rapid, and reversible manner. Figure 4A shows representative confocal micrographs (40X magnification) of rat brain capillaries or microvessels after incubation with 2 μ M NBD-CSA for 30 minutes (steady-state). Treatment with either PSC833 or LPA resulted in a visibly reduced luminal fluorescence (scale bars, 10 μ m). Figure 4B is a graph summarizing the effect of a 30-minute, 0.1-10 nM LPA exposure on specific P-glycoprotein activity, and shows that LPA-elicited decrease in P-glycoprotein transport activity is concentration-dependent. Figure 4C is a time plot showing the combined rapid time-course and reversibility of LPA's action on P-glycoprotein transport activity. After incubation of microvessels to steady-state, LPA was added (t=0) and images were taken at 5, 15, and 30 minutes. At this time-point, LPA was removed and replaced with LPA-free medium (t=30), and images were taken at 35, 45, and 60 minutes. Maximal reduction in specific P-glycoprotein activity by 10 nM LPA is achieved within 30 minutes, and is completely reversible within an additional 30 minutes after experimental treatment (2 μ M NBD-CSA + 10 nM LPA) is replaced with control treatment (2 μ M NBD-CSA only). Each bar depicts the mean value for 10 to 16 microvessels from a single isolation, with tissue pooled from 5 to 10 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$.

Figure 5A-B shows that LPA-elicited reduction in specific P-glycoprotein transport activity is selective for P-glycoprotein activity and does not involve ATP depletion or tight junctional complex disruption. Figure 5A shows that exposure of rat brain capillaries to 10 nM LPA does not alter specific Mrp2 transport activity while exposure to 10 μ M MK571

and 1 mM NaCN reduces specific Mrp2 transport activity maximally by depleting stores ATP stores. The left panel is a graph summarizing these results, whereas the right panel shows representative confocal micrographs of rat brain capillaries incubated for one hour in in one of two media-containing solutions: (1) control: 2 μ M Texas Red only, a fluorescent Mrp2-specific substrate or (2) experimental: 2 μ M Texas Red + 10 nM LPA indicating that accumulation of Texas Red in capillary lumens is unaffected by exposure to 10 nM LPA. Figure 5B shows similar data with the Bcrp transport activity. Figure 5B shows that exposure of rat brain capillaries to 10 nM LPA does not alter specific Bcrp transport activity while exposure to 10 μ M KO143 and 1 mM NaCN reduces specific Bcrp transport activity maximally by depleting stores ATP stores. The left panel shows a graph summarizing the results whereas the right panel shows representative confocal micrographs of rat brain capillaries incubated for one hour in 1) control: 2 μ M BODIPY Prazosin only, a fluorescent Bcrp-specific substrate or (2) experimental: 2 μ M BODIPY Prazosin + 10 nM LPA indicating that accumulation of BODIPY Prazosin in capillary lumens is unaffected by exposure to 10 nM LPA. Each bar depicts the mean value for 10 to 16 microvessels from a single isolation, with tissue pooled from 5 to 10 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$; n.s., not significantly different than control. NaCN, sodium cyanide.

Figure 6A-D shows that LPA reduces specific P-glycoprotein transport activity by ligand activation of the LPA1 Receptor. Figure 6A shows representative confocal micrographs (40X magnification) of rat brain microvessels immunostained for LPA1R and LPA3R. A, abluminal; L, luminal; G, glial/nonvascular. Figure 6B is a graph showing relative abundance of LPA1 Receptor at the apical and basolateral membranes by quantification of fluorescent signal at both. LPA1 Receptor is equally abundant at the apical

and basolateral membranes. Figure 6C is a graph showing P-glycoprotein activity in rat brain capillaries treated with an antagonist for both the LPA1 Receptor and LPA3 Receptor (Ki16425, 100 μ M), a receptor antagonist specific for LPA1 Receptor (AM095, 10 μ M), a receptor agonist specific for LPA1 Receptor (NAEPA, 10 nM) and a receptor agonist specific for LPA3 Receptor ((2S)-OMPT, 10 nM). The LPA1R and LPA1/3R antagonists, AM095 and Ki16425, blocked LPA's action on P-glycoprotein transport activity. The LPA1R agonist NAEPA decreased specific P-glycoprotein transport activity maximally, whereas the LPA3R agonist (2S)-OMPT did not affect P-glycoprotein-mediated transport. Each bar depicts the mean value for 8 to 14 microvessels from a single isolation, with tissue pooled from 3 to 6 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Figure 6D shows that Ki16425, a dual LPA1R-LPA3R antagonist, blocks the LPA-elicited decrease in specific P-glycoprotein transport activity in a concentration-dependent manner. Statistical comparisons: ***, significantly different than control, $p < 0.001$; n.s., not significantly different; $\square\square$, significant difference between treatments, $p < 0.001$.

Figure 7A-E shows that the LPA related reduction in the P-glycoprotein transport activity at the blood-brain barrier occurs in a G-protein-, SRC kinase- and ERK1/2-dependent manner, independently of transcription and translation. Figure 7A is a graph summarizing the P-glycoprotein transport activity in the presence of LPA alone or in the presence of an inhibitor of transcription (actinomycin D, 1 μ M), inhibitor of translation (cycloheximide, 200 μ M), and an inhibitor of G-proteins (GPant-2, 25 μ M). The G-protein antagonist GPant-2 blocked LPA's action on P-glycoprotein transport activity maximally while inhibitors of transcription and translation did not. Figure 7 B shows Western blots for P-glycoprotein after 30-minute exposure of rat brain capillaries to 10 nM LPA indicating that exposure to LPA does not change P-glycoprotein expression or protein levels. Figures

7C and 7D are graphs summarizing the P-glycoprotein transport activity in the presence of LPA alone or in the presence of inhibitors of Src kinase (PP2, 1 μ M) and ERK 1/2 (FR180204, 600 nM) activation respectively. Both a Src kinase inhibitor and an ERK1/2 inhibitor block the action of LPA on P-glycoprotein-mediated transport. Figure 7E shows a schematic of the intracellular signaling events downstream of LPA1R activation and their inhibitors. Each bar depicts the mean value for 8 to 14 microvessels from a single isolation, with tissue pooled from 3 to 6 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$; no sig., no significant difference between treatments; $\square\square$, significant difference between treatments, $p < 0.001$. AMD, Actinomycin D; CHX, Cycloheximide; GPant-2, G-protein Antagonist Peptide-2; P-gp, P-glycoprotein.

Figure 8A-E shows that the tricyclic antidepressant (TCA) amitriptyline (AMT) acts as a ligand at the LPA1 Receptor in rat brain capillaries to maximally reduce specific P-glycoprotein transport activity in a dose-dependent and reversible manner. Figure 8A is a graph showing the specific P-glycoprotein transport activity in the presence of varying concentrations of amitriptyline. Figure 8B is a time plot showing the combined time-course and reversibility assay for amitriptyline. Figure 8C is a graph showing the specific P-glycoprotein transport activity in the presence of amitriptyline alone or in the presence of a receptor antagonist specific for the LPA1 Receptor (AM095, 10 μ M). Figure 8D shows representative confocal micrographs of brain microvessels after incubation with 2 μ M NBD-CSA for 30 minutes. Treatment with AMT resulted in visibly reduced luminal fluorescence, but not in the presence of the LPA1R-specific antagonist AM095 (scale bars, 10 μ m). Figure 8E shows that luminal AMT exposure by perfusion increased the brain accumulation of [3H]-verapamil *in vivo*. Each point on the plot represents the mean value from a cerebral hemisphere, with 4 rats in each treatment group. SEM bars represent experimental

variability, and units are the ratio of disintegrations per minute in the brain to disintegrations per minute in the perfusate (Rbr $\mu\text{L/g}$). Statistical comparisons: ***, significantly different than control, $p < 0.001$; *, significantly different than control, $p < 0.05$.

Figure 9A-C shows that antidepressants, including tricyclic antidepressants (TCAs), decrease P-glycoprotein transport activity. Figure 9A shows that amitriptyline, mianserin, fluoxetine, and nortriptyline decreased P-glycoprotein-mediated efflux in a concentration-dependent manner. Figure 9B depicts the molecular structures of amitriptyline, mianserin, fluoxetine, and nortriptyline. Figure 9C shows that an LPA1R antagonist blocked the actions of amitriptyline, mianserin, and fluoxetine, but not nortriptyline, on P-glycoprotein transport activity. Each bar depicts the mean value for 10 to 14 microvessels from a single isolation, with tissue pooled from 5 to 7 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$; ***, significant difference between treatments, $p < 0.001$; no sig., no significant difference between treatments. AMT, amitriptyline; MSN, mianserin; FLX, fluoxetine; NRT, nortriptyline.

Figure 10A-C shows that amitriptyline and LPA silence ALS-induced P-glycoprotein transport activity. Figure 10A shows representative confocal micrographs (40X magnification) of brain microvessels from either wildtype or human SOD1 transgenic ALS rats. Note the increase in luminal fluorescence seen in microvessels isolated from SOD1 rats compared to wildtype. Figure 10B shows Western blot of rat brain capillary membranes; P-glycoprotein expression is upregulated in human SOD1 transgenic ALS model rats compared to wildtype controls. Figure 10C shows that P-glycoprotein transport activity is significantly increased in SOD1 rats compared to wildtype. Both amitriptyline and LPA reduced P-glycoprotein-mediated transport in either group and this effect was completely blocked by an LPA1R antagonist. Each bar depicts the mean value for 10 to 16

microvessels from a single isolation, with tissue pooled from 5 to 6 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$; $\square\square\square$, significant difference between treatments, $p < 0.001$. WT, wildtype; AMT, amitriptyline.

Figure 11A-D shows that the LPA-elicited decrease in P-glycoprotein transport activity is G-protein-, Src kinase-, and ERK1/2-dependent. (A) Inhibitors of transcription and translation do not block the LPA-elicited decrease in P-glycoprotein transport activity. (B) Western blot of rat brain capillary membranes; 30-minute exposure to 10 nM LPA does not change P-glycoprotein expression or protein levels. (C) LPA does not affect the transport activities of either Mrp2 or Bcrp. (D) A G-protein antagonist (GP2), Src kinase inhibitor (PP2), and ERK1/2 inhibitor (FR 180204) all block the action of LPA on P-glycoprotein-mediated transport, while inhibitors for P13K (LY294002) and the Rho/SRF (CCG1423) pathways do not. Each bar depicts the mean value for 8 to 14 microvessels from a single isolation, with tissue pooled from 3 to 6 rats. SEM bars represent experimental variability, and units are arbitrary fluorescence. Statistical comparisons: ***, significantly different than control, $p < 0.001$. LPA, lysophosphatidic acid; AMD, actinomycin D; CHX, cycloheximide; Bcrp, breast cancer resistance protein; Mrp2, multidrug resistance-associated protein 2; NaCN, sodium cyanide; GP2, G-protein Antagonist Peptide-2; FR, FR180204; LY, LY294002; CCG, CCG1423.

Figure 12 shows the LPA- and amitriptyline-dependent changes in P-glycoprotein-mediated transport at the rat brain microvasculature. Shown are representative confocal micrographs of rat brain microvessels after incubation with 2 μ m NBD-CSA for 30 minutes. Treatment with PSC833, LPA, or AMT results in visibly reduced luminal fluorescence (scale bars, 10 μ m).

DETAILED DESCRIPTION

The present invention is directed at methods and compositions for modulating permeability of a P-glycoprotein expressing barrier of an organ or tissue, such as the blood brain barrier (or BBB), of a subject and/or enhancing or improving delivery of a molecule or an agent across the barrier. For instance, P-glycoprotein present at the blood brain barrier causes a net efflux of its substrate molecules, which include drug or therapeutic or diagnostic molecules, from the BBB and blocks their entry across the BBB. Figure 1 shows a schematic representation of BBB with P-glycoprotein molecules embedded therein. As described herein, administration of ligands that bind to the lysophosphatidic acid Receptor (LPA Receptor or LPAR) selectively and reversibly decreased the transport activity of P-glycoprotein and resulted in increased transport of a co-administered molecule across the blood brain barrier in a rapid and reversible manner.

As described below, it was surprisingly discovered that short-term, low-dose exposure to lysophosphatidic acid (LPA) at endogenous levels selectively and reversibly reduced p-glycoprotein transport activity at the BBB and enhanced or improved or increased the transport of a co-administered molecule across the BBB. (See Example 1). The enhancement or improvement of the transport was rapid and reversible, and did not disrupt or otherwise affect the integrity of the BBB. (See Examples 1 and 2). The reduction in transporter-guided efflux of substrates appeared to occur as a result of rapid signaling mediated by the activity of G-protein coupling (ERK 1/2, and Src kinase dependent) and did not involve the direct degradation of P-glycoprotein, efflux driven by other ABC transporters present at the blood-brain barrier, disruption of microvascular integrity, or metabolic poisoning. (See Examples 3 and 4).

It was further surprisingly discovered that antidepressant molecules, including members of the tricyclic antidepressant (TCA) family of drugs, also achieve reduction in P-glycoprotein transport activity and enhance transport of co-administered molecules in a rapid and reversible manner. (See Examples 5-8). As described in Example 8, amitriptyline exposure improved the delivery of the drug verapamil.

Additionally, it was discovered that the ability of the LPA Receptor ligands to reduce P-glycoprotein-mediated substrate transport is maintained even when the expression of P-glycoprotein is upregulated during disease progression. (See Example 7).

Thus, the present invention provides a novel method of modulation of BBB permeability useful for increasing or improving or enhancing delivery of agents, including drugs or therapeutic or diagnostic molecules, without compromising the inherent neuroprotection afforded by the BBB. Moreover, given that a number of LPA Receptor ligands such as antidepressants, including TCAs, are already approved as being safe for clinical use in humans, the present invention provides a simple and safe method for enhancing or improving drug delivery (or delivery of other agents) to the CNS in humans. The present invention is also applicable in disease states which are characterized by increased expression of P-glycoprotein and exacerbated P-glycoprotein-driven pharmacoresistance.

Accordingly, in one aspect, the present invention includes a method for modulating permeability of the blood brain barrier comprising administering an effective amount of a LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P-glycoprotein.

In another aspect, the present invention includes a method for delivery of an agent across the blood brain barrier of a subject by co-administering the agent with a LPA

Receptor ligand, wherein the LPA Receptor ligand is administered in an amount sufficient to reversibly inhibit the transport activity of P glycoprotein.

In another aspect, the present invention includes a method of treating or preventing or ameliorating a condition or disease by administering to a subject in need thereof a composition which comprises a therapeutic agent effective to treat or prevent or ameliorate the condition or disease and an LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein thereby increasing transport of the therapeutic agent across the blood brain barrier of the subject.

The term "LPA Receptor" refers to a group of G protein-coupled receptors (GPCR) of the bioactive molecule phospholipid lysophosphatidic acid (LPA). Biologic responses to LPA are initiated through binding of LPA to LPA Receptors on the plasma membrane of the cell. Currently at least six different LPA Receptors and their amino acid sequences are known. These include LPA1 Receptor (LPA1R), LPA2 Receptor (LPA2R), LPA3 Receptor (LPA3R), LPA4 Receptor (LPA4R), LPA5 Receptor (LPA5R) and LPA6 Receptor (LPA6R). *See e.g.* the following sequences of LPA receptors isolated from rat and human: GENBANK accession numbers NM_023969.1 and NM_012152.2 (LPAR3), NM_053936.3, NM_057159.2 and NM_001401.3 (LPAR1), NM_001109109.1 and NM_004720.5 (LPAR2), NM_001106940.1, NM_001278000.1 and NM_005296.2 (LPAR4), XM_006225044.3, NM_001142961.1 and NM_020400.5 (LPAR5), and NM_001045843.1, NM_001162498.1, NM_001162497.1 and NM_005767.5 (LPAR6). Based on amino-acid sequence, LPA1R, LPA2R and LPA3R share 50% homology and belong to the EDG subfamily of G-protein coupled receptors formally known as EDG2, EDG4, and EDG7, respectively. The remaining LPA Receptors, including LPAR4, LPAR5 and LPAR6 are structurally distinct from the EDG family and share less than 40% amino-acid sequence homology with the other LPA Receptors.

The term “LPA Receptor ligand” refers to any molecule that binds to a LPA Receptor. In some embodiments the LPA Receptor ligand may bind selectively to only one type of LPA Receptor. For example, in some embodiments the LPA Receptor ligand may bind selectively to LPA1 Receptor (LPA1R), or to LPA2 Receptor (LPA2R), or to LPA3 Receptor (LPA3R), or to LPA4 Receptor (LPA4R), or to LPA5 Receptor (LPA5R), or to LPA6 Receptor (LPA6R) only. In some embodiments the LPA Receptor ligand may bind to more than one type of LPA Receptor. For example, the LPA Receptor ligand may bind to two or three or four or five or six different LPA Receptors. For example, in some embodiments, the LPA Receptor ligand may bind selectively to LPA1 Receptor (LPA1R). In some embodiments, the LPA Receptor ligand may bind selectively to LPA3 Receptor (LPA3R). In some embodiments the LPA Receptor ligand may bind to both LPA1 Receptor (LPA1R) and LPA3 Receptor (LPA3R).

The delivery of the agent according to the present invention results in an enhanced, improved or increased transport of the agent across the BBB. The enhanced, improved or increased transport of the agent refers to an enhancement, improvement or increase in the delivery of the agent across the BBB as compared to the delivery of the agent when administered alone or in the absence of a LPA Receptor ligand. Thus, the amount or concentration of the agent in the CNS when co-administered with the LPA Receptor ligand is higher than the amount or concentration of the agent when administered alone or in the absence of a LPA Receptor ligand. In some embodiments, the delivery of the agent is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or any whole number percentage between 10% and 1000%. In some embodiments, the delivery is increased to about two-fold to about ten-fold of the delivery of the agent in the absence of the LPA Receptor ligand. In some embodiments, the enhanced, improved or increased delivery is at least two-fold,

three-fold, four-fold, five-fold, six-fold, seven-fold, eight-fold, nine-fold, or ten-fold of the delivery of the agent in the absence of the LPA Receptor ligand.

The LPA Receptor ligand is administered in an amount (or at a concentration) sufficient to reversibly inhibit (or reduce) the transport activity of P glycoprotein. The inhibition or reduction of the transport activity of P-glycoprotein may be by at least about 5% to about 100%, or any whole number percentage between 5% and 100%, of the transport activity of P-glycoprotein in the absence of administration of the LPA Receptor ligand. For example, the inhibition or reduction of the transport activity of P-glycoprotein may be by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%. The inhibition or reduction of the P-glycoprotein is rapid and reversible.

Without wishing to be bound by theory, it is believed that the inhibition or reduction of the transport activity P glycoprotein may be primarily due to selective activation of LPA1 Receptor present at the BBB by the LPA1 Receptor ligand and occurs as a result of rapid signaling mediated by the activity of G-protein coupling and does not involve the direct degradation of P-glycoprotein, any efflux driven by other ABC transporters present at the blood-brain barrier, disruption of microvascular integrity, or metabolic poisoning.

Thus, in some embodiments, the transport of the agent during the method of the present invention does not disrupt or affect the integrity or physical permeability of the BBB, or does not alter substantially the physical structure of the brain capillaries that contribute to barrier function or the tight junctions to make the capillaries become more leaky, or does not cause the lipid bilayer to become more permeable. Without wishing to be bound by theory, it is submitted that signaling through the LPA receptor alters the activity of a biological pump that regulates the entry of specific molecules (substrates) to the brain.

In some embodiments, the LPA Receptor ligand may be a LPA1 Receptor ligand. In some embodiments, the LPA Receptor ligand is a selective ligand for LPA1 Receptor, and does not bind to other LPA Receptors. In some embodiments, the LPA Receptor ligand may bind to another LPA Receptor in addition to the LPA1 Receptor. For example, it may bind to LPA1 Receptor and LPA3 Receptor. In some embodiments, a combination of LPA Receptor ligands may be used. For example, in some embodiments a mixture of two or more LPA Receptor ligands that bind to LPA1 Receptor may be used. In some embodiments a mixture of two or more LPA Receptor ligands, that bind to two different LPA Receptors may be used.

In some embodiments the LPA Receptor ligand may be LPA or a structural analog thereof. The structure of LPA is shown in Figure 2A. Examples of suitable LPA Receptor ligands include, without limitation, LPA, NAEPA, OMPT, oleoyl-thiophosphate, 2-oleoyl-LPA, and T13.

In some embodiments, the LPA Receptor ligand may be an antidepressant. In some embodiments the LPA Receptor ligand may be a tricyclic antidepressant (TCA). The structure of a TCA is shown in Figure 2B. A number of TCAs are known in the art, some of which have been approved for clinical use, and are included herein. Examples include, without limitation, amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, and amoxapine. In some embodiments, the TCA may be amitriptyline, nortriptyline, mianserin or a combination thereof. In some embodiments, the TCA may be amitriptyline. In some embodiments, the LPA Receptor ligand may be an antidepressant that is not a TCA, such as fluoxetine.

It should be appreciated that while in some embodiments of the invention, the LPA Receptor ligand can be a TCA, the TCA is not being administered to a patient to achieve an anti-depression therapeutic effect but rather to achieve the physiological result of improved

transport of a co-administered agent across the blood brain barrier of a subject. Thus, in embodiments of the invention in which the LPA Receptor ligand is a TCA, the patient may be a patient that has not been diagnosed with depression and/or does not have symptoms of depression. Alternatively, the patient may have been diagnosed with depression and/or have symptoms of depression but is being administered a TCA as part of a regimen for improving transport of an agent across the blood brain barrier. Such patients may be receiving alternative therapies for treatment of depression such as pharmacologic therapies or psychiatric therapies or may be receiving no treatment at all of depression.

In some embodiments the LPA Receptor ligand may be administered as a prodrug, which after administration is metabolized into an active form that binds to a LPA Receptor.

The term "agent" refers to any compound or molecule that is known or thought to be a substrate of P-glycoprotein. It may be a therapeutic or a diagnostic agent or any other compound of interest.

A "therapeutic agent" means any compound or molecule having a biological activity that is known or thought to be a substrate of P-glycoprotein. Therapeutic agents encompass the full spectrum of treatments for a condition or disease or disorder. A therapeutic agent may act in a manner that is prophylactic or preventive, including those that incorporate procedures designed to target individuals that can be identified as being at risk (pharmacogenetics); or in a manner that is ameliorative or curative in nature; or may act to slow the rate or extent of the progression of a condition or disease or disorder; or may act to minimize the time required, the occurrence or extent of any discomfort or pain, or physical limitations associated with recuperation from a condition, disease, disorder or physical trauma; or may be used as an adjuvant to other therapies and treatments. The terms "treatment," "treating," and the like mean obtaining a desired pharmacologic and/or physiologic effect. Treatment includes inhibiting a condition or disease, (e.g., arresting its

development) and relieving a condition or disease (e.g., reducing symptoms associated with a condition or disease). Treatment as used herein covers any administration of a pharmaceutical agent or compound to an individual to treat, cure, alleviate, improve, diminish, or inhibit a condition in the individual. The term “treating prophylactically” means
5 reducing the frequency of occurrence of a condition or disease or the severity of the condition or disease by administering an agent prior to appearance of a symptom of that condition or disease. The prophylactic treatment may completely prevent or reduce appearance of the condition or disease or a symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a condition or disease and/or adverse effect
10 attributable to the condition or disease. Prophylactic treatment may include reducing or preventing a disease or condition (e.g., preventing cancer) from occurring in an individual who may be predisposed to the condition or disease but has not yet been diagnosed as having it.

Examples of therapeutic agents include, without limitation, anti-bacterial agents,
15 anti-viral agents, anti-mycotic agents, anti-cancer agents, analgesics, chemotherapeutic agents, drugs, and other molecules or medicines or compounds that are known or thought to be substrates of P-glycoprotein. Upon administration, the therapeutic agent may localize to a particular part of the brain or perfuse generally once it crosses the BBB.

A “diagnostic agent” means any compound or molecule useful in diagnostic
20 applications that is known or thought to be a substrate of P-glycoprotein. Examples of diagnostic agents include imaging molecules or labeling molecules, for example, substances that are labeled with a radioactive label, or fluorescent label or substances used in magnetic resonance imaging (MRI) procedures. Detectable labels, or markers, for use in the present invention may be a radiolabel, a fluorescent label, a nuclear magnetic resonance active label,
25 a luminescent label, a chromophore label, a positron emitting isotope for PET scanner, or a

chemiluminescence label. Upon administration, the diagnostic agent may localize to a particular part of the brain or perfuse generally once it crosses the BBB.

The concentration or amount of LPA Receptor ligand that is “sufficient to reversibly inhibit transport activity of P glycoprotein” will depend on the route of administration and the dosage form administered and can be determined by one of skill in the art through routine experimentation using methods described herein and known in the art.

In some embodiments, the amount of the LPA Receptor ligand administered may be sufficient to achieve a blood concentration level that is in the range of 0.1 nM to 1000.0 nM. Other ranges for the blood concentration level are also included in the present invention.

For instance, in some embodiments, a lower endpoint of a range may be selected from 0.1 nM, 0.2 nM, 0.3 nM, 0.4 nM, 0.5 nM, 0.6 nM, 0.7 nM, 0.8 nM, 0.9 nM, 1 nM, 2 nM, 3 nM, 4 nM, 5 nM, 6 nM, 7 nM, 8 nM, 9 nM, 10 nM, 15 nM, 20 nM, 25 nM, 30 nM, 35 nM, 40 nM, 45 nM, 50 nM, 55 nM, 60 nM, 65 nM, 70 nM, 75 nM, 80 nM, 85 nM, 90 nM, 95 nM, 100 nM, 150 nM, 200 nM, 250 nM, 300 nM, 350 nM, 400 nM, 450 nM, 500 nM, 550 nM, 600 nM, 650 nM, 700 nM, 750 nM, 800 nM, 850 nM, or 900 nM (or any number in between), and an upper endpoint of a range may be selected from 900 nM, 850 nM, 800 nM, 750 nM, 700 nM, 650 nM, 600 nM, 550 nM, 500 nM, 450 nM, 400 nM, 350 nM, 300 nM, 250 nM, 200 nM, 150 nM, 100 nM, 95 nM, 90 nM, 85 nM, 80 nM, 75 nM, 70 nM, 65 nM, 60 nM, 55 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM or 1 nM (or any number in between). In some embodiments, the amounts or concentrations of an antidepressant, including a TCA, sufficient to reversibly inhibit transport activity of P glycoprotein may be 10-1000 fold less than those required in standard depression therapy. As noted before, in some embodiments, a combination of two or more LPA Receptor ligands may also be used. The total concentration of LPA Receptor ligand administered may be < 1 μ M. The term “subject” includes any subject which possesses a blood brain barrier and

includes any mammal such as humans or non-human mammal such as horses, dogs, cats, pigs, goats, rabbits, hamsters, monkeys, guinea pigs, rats, mice, etc. In one embodiment, the subject is a human.

The methods of the present invention can be used not only to deliver the agents to a particular site of administration, but also provide systemic delivery. Further, since P-glycoprotein is also expressed at the barriers of other organs or tissues including kidney, liver, intestine, retina, placenta and testes, the present invention also includes uses to enhance delivery at these locations. Furthermore, since P-glycoprotein functions to remove (excretion) drugs from the body, for example in the kidney and liver, inhibition of its activity is useful for maintaining higher serum levels for the drug by inhibiting removal of the drug from the body.

The methods and compositions of the present invention are useful in treating a variety of diseases or medical conditions by improving the delivery of a therapeutic agent across the BBB (as well as barriers at other organs or tissues, including kidney, liver, intestine, retina, placenta and testes, as discussed above), which not only improves the efficaciousness of a drug but also reduces the amount of drug required to be administered thus reducing its side effects.

In some embodiments, due to the improved transport of the agent across the BBB and improved delivery of the agent to the CNS, the dosage required of an agent to achieve its therapeutic or diagnostic effect would typically be lower when co-administered with the LPA Receptor ligand than the dosage required in the absence of the LPA Receptor ligand. For example, in some embodiments, due to the improved delivery of the agent, it is possible to achieve at least five-fold, six-fold, seven-fold, eight-fold, nine-fold, ten-fold, or at least 100-fold, or at least 1000-fold reduction (and including any whole number-fold reduction between 10 and 1000) in the dosage of the therapeutic or diagnostic agent to be

administered, thus improving the efficacy of the agent. In some embodiments, due to the improved transport of the agent across the BBB and improved delivery of the agent to the CNS, the frequency of administration of an agent to achieve its therapeutic or diagnostic effect would typically be lower when co-administered with the LPA Receptor ligand than the frequency required in the absence of the LPA Receptor ligand.

Due to a reduction in the dosage and/or frequency of administration of the agent, less side effects from administration of the agent may be seen. For example, in some embodiments, the agent may be a chemotherapeutic drug. Co-administering the chemotherapeutic drug with a LPA Receptor ligand in accordance with the method of the present invention, may serve to reduce the dosage of chemotherapeutic drug administered to a patient by between about 10-1000 fold. Additionally, or alternatively it may also reduce the frequency of administration of the chemotherapeutic drug administered. A reduction in the dosage and/or frequency of chemotherapy would reduce many of the side-effects typically seen with such drugs.

In some embodiments of the present invention, where the LPA Receptor ligand is a TCA, the TCA is administered with the agent as per the dosing schedule of the agent. Such administration of a TCA as per the dosing schedule of the agent to improve the transport of the agent across the BBB is different from the administration of a TCA for treating psychiatric disorders. For example, a TCA is often administered chronically for treating depression. In the methods of the present invention, if the agent is administered on an as-needed or PRN basis, then the TCA is also administered on as-needed or PRN basis along with the agent.

The LPA Receptor ligand and the agent may be administered concurrently or sequentially, provided that the LPA Receptor ligand is administered close enough in time to the agent administration to achieve a therapeutically significant increase in transport of the

agent across the BBB (or barriers of other organs or tissues as discussed above). They may be administered simultaneously as a combination, or concurrently but separately. They may be combined with other compounds or therapies. Administration may be by any suitable route, for example, by a enteral route including oral (by mouth), by a feeding tube, rectal
5 (suppository) and such, or by a parenteral route including intravenous, subcutaneous, transdermal, intramuscular and such, or by a topical route including epicutaneous, inhalational, nasal, eye drops, ear drops and such.

Further embodiments of the invention include co-formulations of an LPA Receptor ligand and an agent in which the two components are in physical admixture, such as being
10 in a dosage formulation in a tablet, pill, capsule or other solid formulation. Alternatively, such a co-formulation can include a liquid formulation such as in an IV bag. In such co-formulations, the LPA Receptor ligand is present in amounts or concentrations sufficient to improve transport of the agent across the BBB, and the agent is present in amounts or concentrations within the therapeutic window, accounting for the improved transport of the
15 agent across the BBB. That is, the amount of the agent in the co-formulation would be less than in a formulation of the same agent in the absence of an LPA Receptor ligand.

Compositions can be administered in solid dosage forms, such as tablets, pills, capsules, cachets, granules and powders; or in liquid forms such as elixirs, syrups, solutions, suspensions, emulsions or microemulsions. They may be administered as bolus, electuary
20 or paste. Dosage forms for the topical or transdermal administration of compounds of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. Transdermal patches have the added advantage of providing controlled delivery of compounds of the invention to the body.

These compositions may also contain pharmaceutically acceptable excipients and
25 adjuvants. It may also be desirable to include isotonic agents, such as sugars, sodium

chloride, and the like in the compositions. Suitable pharmaceutically acceptable carriers are well known in the art. Descriptions of some of these pharmaceutically acceptable carriers may be found in The Handbook of Pharmaceutical Excipients, published by the American Pharmaceutical Association. Methods of formulating pharmaceutical compositions have
5 been described in numerous publications such as Pharmaceutical Dosage Forms: Tablets, Second Edition, Revised and Expanded, Volumes 1-3, edited by Lieberman et al; Pharmaceutical Dosage Forms: Parenteral Medications, Volumes 1-2, edited by Avis et al; and Pharmaceutical Dosage Forms: Disperse Systems, Volumes 1-2, edited by Lieberman et al; published by Marcel Dekker, Inc.

10 The compositions and formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

15 Examples of the diseases or medical conditions encompassed in the present invention include neurological diseases, cancer, pain, cardiovascular diseases, and autoimmune diseases. Examples of cancer include without limitation, brain cancer, neuroma, glioma, lymphoma, and glioblastoma. Examples of a neurological disease include without limitation brain tumor, brain metastasis, schizophrenia, epilepsy, Parkinson's
20 disease, Huntington's disease, stroke, a disease associated with malfunction of the BBB, a neurodegenerative disease such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, dementia, and HIV-associated dementia (known as HAD or *AIDS* dementia complex, ADC). Examples of pain include centrally mediated pain, peripherally mediated pain, neuropathic pain, acute pain, and chronic pain.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art. Such techniques are explained fully in the literature, such as, *Methods of Enzymology*, Vol. 194, Guthrie et al., eds., Cold Spring Harbor Laboratory Press (1990); *Biology and activities of yeasts*, Skinner, et al., eds., Academic Press (1980); *Methods in yeast genetics : a laboratory course manual*, Rose et al., Cold Spring Harbor Laboratory Press (1990); *The Yeast Saccharomyces: Cell Cycle and Cell Biology*, Pringle et al., eds., Cold Spring Harbor Laboratory Press (1997); *The Yeast Saccharomyces: Gene Expression*, Jones et al., eds., Cold Spring Harbor Laboratory Press (1993); *The Yeast Saccharomyces: Genome Dynamics, Protein Synthesis, and Energetics*, Broach et al., eds., Cold Spring Harbor Laboratory Press (1992); *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook et al., 1989) and *Molecular Cloning: A Laboratory Manual*, third edition (Sambrook and Russel, 2001), (jointly referred to herein as “Sambrook”); *Current Protocols in Molecular Biology* (F.M. Ausubel et al., eds., 1987, including supplements through 2001); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York; Harlow and Lane (1999) *Using Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (jointly referred to herein as “Harlow and Lane”); Beaucage et al. eds., *Current Protocols in Nucleic Acid Chemistry* John Wiley & Sons, Inc., New York, 2000); Casarett and Doull’s *Toxicology The Basic Science of Poisons*, C. Klaassen, ed., 6th edition (2001), and *Vaccines*, S. Plotkin and W. Orenstein, eds., 3rd edition (1999).

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those

skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

The following examples are provided for the purpose of illustration and are not intended to limit the scope of the present invention. Each publication, sequence or other reference disclosed below and elsewhere herein is incorporated herein by reference in its entirety, to the extent that there is no inconsistency with the present disclosure.

Examples:

The following materials and methods were used in the examples.

Materials. 1-Oleoyl-lysophosphatidic acid (LPA) was purchased from Santa Cruz Biotechnology. Mouse monoclonal P-glycoprotein antibody was obtained from Covance. Secondary goat anti-rabbit IgG (H+L) Alexa Fluor 488 antibody was purchased from Invitrogen. The specific P-glycoprotein inhibitor PSC 833, Mrp2 inhibitor MK 571, Bcrp inhibitor KO 143, ERK 1/2 inhibitor FR 180204, and Src kinase inhibitor PP2 were all purchased from Tocris Bioscience. The antagonist for LPA1R and LPA3R Ki16425, agonist for LPA3R (2S)-OMPT, and Rabbit polyclonal LPA1R antibody were obtained from Cayman Chemical. The antagonist for LPA1R AM095 was purchased from ApexBio. The fluorescent P-glycoprotein substrate N-ε(4-nitrobenzofurazan-7-yl)-D-Lys⁸ cyclosporin A (NBD-CSA) was custom-synthesized, the fluorescent Bcrp substrate BODIPY® FL prazosin was purchased from Invitrogen, and the fluorescent Mrp2 substrate sulforhodamine 101 free acid (Texas Red) was purchased from Sigma-Aldrich. Mouse monoclonal β-actin antibody, specific LPA1R agonist NAEPA, amitriptyline hydrochloride, mianserin hydrochloride, fluoxetine hydrochloride, nortriptyline hydrochloride, Actinomycin D, Cycloheximide, Ficoll, and all other chemicals were all also purchased from Sigma-Aldrich.

Animals. All experiments were executed in accordance with the National Institutes of Health animal care and use guidelines and approved by the National Institute of Environmental Health Sciences (NIEHS) Animal Care and Use Committee. Adult male Sprague Dawley rats between the ages of six and nine months were obtained from Taconic. Human SOD1 transgenic ALS model rats were also obtained from Taconic. All animals were housed in temperature- and humidity-controlled rooms with a 12-hour light/dark cycle and *ad libitum* access to food and water. Animals were killed by carbon dioxide inhalation, followed by immediate decapitation. SOD1 rats were collected after disease presentation, indicated by the development of limb paralysis with simultaneous weight loss indicative of muscle wasting.

Example 1 illustrates that short-term, low-dose LPA exposure reduces specific p-glycoprotein transport activity at the BBB rapidly, reversibly and in a dose-dependent manner.

An established confocal imaging based assay was used to determine the transport activity of P-glycoprotein in freshly isolated rat brain capillaries, which constitute the microvascular component of the blood-brain barrier *ex vivo*. Brain capillaries were isolated and either immediately used for transport assay and immunohistochemistry studies or prepared and frozen for additional Western blotting analysis. This scheme is shown in Figure 3. The assay and resulting analyses rely first on the accumulation of the specific fluorescent P-glycoprotein substrate N-ε(4-nitrobenzofurazan-7-yl)-D-Lys⁸ cyclosporin A (NBD-CSA) within the luminal space of each capillary. The methods used are described in detail below.

Capillary isolation. Procedures for capillary isolation were outlined previously (14-16). The step involving syringe column passage has been replaced with one utilizing 30 μm cell strainers (pluriSelect). After decapitation, rat brains were transferred to cold PBS

(in mM: 2.7 KCl, 1.5 KH₂PO₄, 136.9 NaCl, 8.1 Na₂HPO₄, 1 MgCl₂, 1 CaCl₂, supplemented with 5 D-glucose, 1 sodium pyruvate, pH 7.4). Tissue was kept in cold PBS for the remainder of the capillary isolation process. White matter, meninges, midbrain, choroid plexus, blood vessels, and olfactory lobes were dissected away and remaining brain tissue was homogenized. An aliquot of 30% Ficoll was added to an equivalent volume of brain homogenate and capillaries were separated from remaining brain parenchyma by centrifugation (5800g, 20 minutes). Capillary pellets were washed with 1% bovine serum albumin in PBS and passed through a series of 30 µm cell strainers (pluriSelect), washed sequentially with PBS, and used immediately.

Transport Assay. Confocal microscopy- and imaging-based transport assays with isolated rat brain capillaries were conducted as characterized previously (16). All transport assay studies were conducted at room temperature within coverslip-bottomed imaging chamber slides filled with PBS. The fluorescent substrates, NBD-CSA for P-glycoprotein, Texas Red for Mrp2, and BODIPY® FL prazosin for Bcrp, were added with or without either LPA or one of four antidepressant drugs and luminal substrate accumulation was measured at varying time-points in the presence or absence of different antagonists, agonists, and inhibitors. In every study, a specific inhibitor (PSC 833 for P-glycoprotein, MK 571 for Mrp2, and KO 143 for Bcrp) was also added in order to determine the specific component of luminal substrate accumulation that was transport-mediated. Capillaries were imaged with a Zeiss 510-inverted confocal laser-scanning microscope through a 40 × water-immersion objective (numeric aperture of 1.2) using a 488 nm laser line for both NBD-CSA and BODIPY® FL prazosin and a 543 nm laser line for Texas Red. Images were saved to a thumb drive, transferred, and luminal fluorescence was quantitated using NIH Image J software as characterized previously (11). Data reported are for a single study that is

representative of three to five experimental replicates. Detailed methods to isolate and conduct transport experiments are described in Chan and Cannon (2017).

Immunostaining for LPA1R, LPA3R, and P-glycoprotein. Isolated brain capillaries were fixed, permeabilized, and blocked in PBS as outlined previously (17). Capillaries were then incubated at 4° C overnight in either the primary rabbit polyclonal LPA1R antibody, primary rabbit polyclonal LPA3R antibody, or primary mouse monoclonal P-glycoprotein antibody and then at room temperature for ninety minutes in either the secondary goat anti-rabbit IgG (H+L) Alexa Fluor 488 antibody or goat anti-mouse fluorescent dye IRDye 800CW. The primary antibodies used recognized either LPA1R or LPA3R, membrane-localized G-protein coupled receptors, or the transmembrane ABC transporter P-glycoprotein. Immunostained capillaries were imaged with a Zeiss 510-inverted confocal laser-scanning microscope through a 40× water-immersion objective (numeric aperture of 1.2) using a 488 nm laser line. Images were saved to a thumb drive, transferred, and fluorescence at the luminal and abluminal membranes was determined using NIH ImageJ software.

Western blotting. Endothelial membranes were isolated from control and LPA-exposed capillaries as outlined previously (18-19). Membrane protein was assayed by the Bradford method and an aliquot of the membrane protein determined from the Bradford-derived standard curve was mixed with NuPAGE 4 × sample buffer obtained from Invitrogen. The mixture was loaded onto a 4-12% Bis-Tris NuPAGE gel, electrophoresed, and subsequently transferred to an Immobilon-FL membrane obtained from Millipore. Odyssey Blocking Buffer from Li-Cor Biosciences was added to the membrane at room temperature for forty-five minute to block, after which the membrane was immunoblotted with mouse monoclonal P-glycoprotein antibody (170 kDa, 1:200). The membrane was then stained with goat anti-mouse fluorescent dye IRDye 800CW (1:1000) in PBS at room

temperature for two hours and sequentially washed in 0.05% Tween in PBS. The membrane was imaged using an Odyssey Infrared Imaging System from Li-Cor Biosciences. β -actin (42 kDa, 1:10,000) was used as a loading control.

Statistical Analyses. Quantitative data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses of differences between experimental groups were performed by one-way ANOVA (Tukey multiple comparison test) using Prism 6.0 software. Differences between experimental group means were considered significant when $p < 0.05$.

Figure 4A includes representative confocal micrographs of freshly isolated rat brain capillaries incubated to steady-state in one of three conditions: (1) medium containing only 2 μ M NBD-CSA to represent control or baseline levels of luminal substrate accumulation, (2) medium containing both 2 μ M NBD-CSA and 10 μ M PSC 833, a specific P-glycoprotein inhibitor, and (3) medium containing both 2 μ M NBD-CSA and 10 nM LPA, an endogenous phospholipid. Luminal fluorescence was visibly reduced in confocal micrographs of capillaries exposed to either PSC 833 or LPA when compared to control. LPA exposure resulted in a loss of luminal NBD-CSA accumulation, as measured by fluorescence intensity, comparable to that elicited by PSC 833, which is considered to be both maximal and specific. To this end, only the component of luminal NBD-CSA accumulation that is sensitive to PSC 833 treatment was considered to be P-glycoprotein-mediated and used to measure specific P-glycoprotein transport activity, as done previously. Exposing brain capillaries to 0.1-10 nM LPA for thirty minutes reduced specific P-glycoprotein transport activity in a dose-dependent manner (Figure 4B).

In order to determine whether this reduction in transport activity was transient or sustained post-exposure, a combined time-course and reversibility assay was performed. Brain capillaries were incubated in media containing 10 nM LPA for five, fifteen, and thirty minutes, at which point these solutions were replaced with media containing only 2 μ M

NBD-CSA and allowed to incubate for a further five, fifteen, and thirty minutes. Confocal micrographs were obtained for each of these time points, and the results are shown in Figure 4C. Thirty-minute exposure of brain capillaries to 10 nM LPA resulted in maximal reduction in specific P-glycoprotein transport activity, which returned to levels recorded at the pre-exposure time-point within thirty minutes after removal of LPA.

Thus, the LPA-elicited decrease in specific P-glycoprotein transport activity is maximal, rapid, and reversible, modeling what might occur at the blood-brain interface within the context of rapid circulation and blood clearance when LPA is either released endogenously or administered exogenously.

Example 2 illustrates that LPA exposure selectively reduces p-glycoprotein transport activity.

Because the LPA-elicited reduction in luminal NBD-CSA accumulation could be non-specific and result from either gross leakiness or metabolic poisoning, the effect of 10 nM LPA exposure on the specific transport activities of two other ABC transporters present at the blood-brain barrier, Mrp2 and Bcrp, was assessed using the materials and methods described above.

As shown in Figure 5, exposing brain capillaries to 10 nM LPA did not alter the steady-state luminal accumulation of either of the fluorescent molecules sulforhodamine 101 free acid (Texas Red) or BODIPY® FL prazosin, which are specific substrates for the ABC transporters Mrp2 (Figure 5A) and Bcrp (Figure 5B), respectively. Treatment with sodium cyanide reduced the luminal accumulation of both Texas Red and BODIPY® FL prazosin by depleting ATP stores. See also Figure 11 C. Any disruption of tight junctional complex integrity would have resulted in a reduced luminal Texas Red accumulation, as shown in studies where rat brain capillaries were incubated in hyperosmotic media containing 100 mM sucrose.

Because exposure of brain capillaries to LPA had no effect on the luminal accumulation of specific substrates for Mrp2 and Bcrp, it was concluded that the LPA-elicited decrease in luminal NBD-CSA accumulation is specific to the transport activity of P-glycoprotein and does not involve the disruption of either tight junctional complex integrity or ATP availability.

Example 3 illustrates that LPA reduces specific p-glycoprotein transport activity primarily in a LPA1 Receptor-mediated manner.

LPA signals through a family of six GPCRs, the expression profiles of which are tissue-specific. Immunostaining for LPA1 Receptor and LPA3 Receptor with receptor-specific antibodies and immunofluorescence indicates expression of LPA1 Receptor, but not LPA3 Receptor, in rat brain capillaries at both the apical (inner) and basolateral (outer) membranes. LPA1 Receptor is equally abundant at the apical and basolateral membranes (see Figures 6A and 6B). While LPA1R was detected at both the luminal and abluminal membranes of rat-derived CNS microvessels, LPA3R was detected in extravascular supporting cells that also traverse the capillary isolation process, which coincides with previous studies that report its expression in astrocytes and microglia.

Brain capillaries were exposed to an antagonist of LPA1R and LPA3R both, an antagonist of LPA1R only, an agonist of LPA1R only and an agonist of LPA3R only. Treatment of rat brain capillaries with Ki16435, 100 μ M (antagonist for both the LPA1 Receptor and LPA3 Receptor) blocked the reduction in specific P-glycoprotein activity resulting from exposure to LPA in a concentration dependent manner (see Figure 6D). Treatment of rat brain capillaries with AM095, 10 μ M (a receptor antagonist specific for LPA1 Receptor only) also completely blocked the reduction in specific P-glycoprotein transport activity resulting from exposure to LPA (see Figure 6C). Further, treatment with 10 nM (2S)-OMPT, a specific LPA3R agonist, failed to reduce luminal NBD-CSA

accumulation while treatment with 10 nM NAEPA, a specific LPA1R agonist, did so maximally (Figure 6C). Taken together, these data indicate that LPA reduces specific P-glycoprotein transport activity primarily through activation of LPA1R.

Example 4 illustrates that LPA signals to reduce specific P-glycoprotein transport activity at the blood-brain barrier in a G-protein and ERK1/2-dependent manner, independently of transcription and translation.

Because the reduction in specific P-glycoprotein transport activity caused by LPA exposure is reversible and effectively blocked by antagonizing LPA1R, the intracellular signaling events downstream of LPA1R activation were investigated. A schematic of the pathway and its inhibitors is shown in Figure 7E.

Treatment of rat brain capillaries with inhibitors of transcription (actinomycin D or AMD, 1 μ M) and translation (cycloheximide or CHX, 200 μ M) did not block the reduction in specific P-glycoprotein transport activity resulting from exposure to LPA. Treatment of rat brain capillaries with an inhibitor of G-proteins (GPAnt-2, 25 μ M), however, completely blocked the reduction in specific P-glycoprotein transport activity resulting from exposure to LPA (see Figure 7A). Western blotting for P-glycoprotein after 30-minute exposure of rat brain capillaries to 10 nM LPA indicated no change in levels of P-glycoprotein (see Figure 7B). See also Figure 11A, B and D.

Treatment of rat brain capillaries with inhibitors of Src kinase and ERK 1/2, intracellular kinases that are involved in a variety of signaling cascades, blocked the reduction in specific P-glycoprotein activity resulting from exposure to LPA. Treatment with 1 μ M PP2 and 600 nM FR180204, inhibitors of Src kinase and ERK 1/2 activation respectively, maximally blocked the reduction in specific P-glycoprotein transport activity downstream of LPA1R activation by LPA (Figure 7C and 7D for Src kinase and ERK1/2 respectively, See also Figure 11D); however, treatment with either 1 μ M LY294002 or 300

nM CCG1423, inhibitors of PI3K and the Rho/SRF pathway respectively, did not (Figure 11D).

These results show that the reduction in specific P-glycoprotein transport activity occurs independently of expression (transcription or translation) or degradation of the protein, that LPA elicits its effect on P-glycoprotein transport activity by a rapid signaling cascade that depends on the ability of GPCRs to couple to their respective G-proteins, and that the activities of G-proteins, Src kinase, and ERK 1/2, but not of PI3K or Rho/SRF, are required for the decrease in P-glycoprotein-mediated transport downstream of LPA1R at the rat brain microvasculature.

10 Example 5 illustrates that the tricyclic antidepressant (TCA) amitriptyline activates LPA1R in rat brain capillaries to reduce the specific transport activity of P-glycoprotein in a dose-dependent and reversible manner.

Amitriptyline exposure for 30 minutes at concentrations ranging from 0.1 pm -10 nM reduced specific P-glycoprotein transport activity in a dose-dependent manner, and 15 maximal reduction in specific P-glycoprotein activity was achieved at a concentration of 10 nM (Figure 8A). A combined time-course and reversibility assay demonstrated that maximal reduction in specific P-glycoprotein activity by 10 nM amitriptyline was achieved within 30 minutes, and was completely reversible within an additional 30 minutes after experimental treatment (2 μ M NBD-CSA + 10 nM amitriptyline) is replaced with control 20 treatment (2 μ M NBD-CSA only) (Figure 8B). Further, treatment of rat brain capillaries with a receptor antagonist specific for the LPA1 Receptor (AM095, 10 μ M) completely blocked the reduction in specific P-glycoprotein transport activity resulting from exposure to amitriptyline (Figure 8C).

Figure 8D shows representative confocal micrographs of brain microvessels after 25 incubation with 2 μ M NBD-CSA for 30 minutes. Treatment with amitriptyline (AMT)

resulted in visibly reduced luminal fluorescence, but not in the presence of the LPA1R-specific antagonist AM095.

These results show that amitriptyline acts as a ligand at the LPA1 Receptor in rat brain capillaries to reduce specific P-glycoprotein transport activity in a dose-dependent and reversible manner.

Example 6 illustrates that antidepressants such as fluoxetine, amitriptyline, mianserin, and nortriptyline reduce the specific transport activity of P-glycoprotein *ex vivo*.

Exposing rat brain capillaries to 0.1-10 nM of amitriptyline, mianserin, and nortriptyline and fluoxetine for thirty minutes reduced specific P-glycoprotein transport activity in a dose-dependent manner, although to varying degrees compared to that elicited by exposure to LPA (Figure 9A). Structures of these molecules are shown in Figure 9B.

Treatment of brain capillaries with 10 μ M AM095 which is a LPA1R antagonist completely blocked the reduction in specific P-glycoprotein transport activity elicited by amitriptyline, mianserin, and fluoxetine, but not by nortriptyline (Figure 9C). These data indicate that amitriptyline, mianserin, and fluoxetine, but not nortriptyline, mimic LPA at the blood-brain barrier by activating LPA1R to reduce the specific transport activity of P-glycoprotein *ex vivo* (Figure 9C).

Example 7 illustrates that LPA and amitriptyline activate LPA1R to reduce the specific transport activity of P-glycoprotein even when induced as a component of a disease process.

The upregulation of P-glycoprotein expression and transport activity in SOD1 transgenic animals has been reported previously, implicating P-glycoprotein in the phrmacoresistance associated with ALS disease progression. Figure 10A includes representative confocal micrographs of brain capillaries isolated from human SOD1 transgenic ALS model and age-matched wildtype rats incubated to steady-state in media containing only 2 μ M NBD-CSA. Luminal substrate accumulation is clearly increased in

capillaries isolated from SOD1 rats compared to their wildtype counterparts. P-glycoprotein upregulation was confirmed in SOD1 rats compared wildtype, and the presence of LPA1R was detected in both rat models using protein-specific antibodies and immunofluorescence (Figure 10B). Treatment of CNS microvessels derived from human SOD1 transgenic ALS model rats with either 10 nM LPA or 10 nM amitriptyline reduced specific P-glycoprotein transport activity to levels seen in CNS microvessels from wildtype rats, and this reduction was completely blocked by treatment with 10 μ M AM095 (Figure 10C). Taken together, these data indicate that LPA and amitriptyline activate LPA1R in human SOD1 transgenic ALS model rat capillaries to reduce the specific transport activity of P-glycoprotein, even when induced as a component of a disease process.

Example 8 illustrates that luminal amitriptyline exposure increases the brain accumulation of [3H]-verapamil.

Since *ex vivo* exposure does not allow for the luminal- or abluminal-specific receptor activation, *in situ* brain perfusion was utilized as an *in vivo* model to observe changes in P-glycoprotein transport activity given luminal amitriptyline exposure.

Verapamil is a therapeutic drug molecule which works by relaxing muscles of the heart and blood vessels, and is a glycoprotein substrate. Brain perfusion experiment was executed as described previously (20). Rats were anesthetized with a 1 mL/kg ketamine mixture (79 mg/mL ketamine, 3 mg/mL xylazine, 0.6 mg/mL acepromazine) and administered heparin (10 kU/kg) via intraperitoneal injection. After exposing the common carotid arteries by midline incision at the neck, the common carotid arteries were perfused with oxygenated Ringer's solution at 37 °C (in mM, 117 NaCl, 4.7 KCl, 0.8 MgSO₄, 24.8 NaHCO₃, 1.2 KH₂PO₄, 2.5 CaCl₂, 10 D-glucose, in g/L, 39 dextran, 1 BSA and 0.055 Evans blue) at 3 mL/min. [3H]-verapamil (0.1 μ Ci/mL) was infused into the circuit via syringe pump at 0.5 mL/min for 20 min. Samples of perfusate were collected from the cannulae at

the end of each experiment. Brains were removed, separated by hemisphere, stripped of meninges, midbrain, and choroid plexuses, and minced. Tissue and 100 μ L perfusate samples were solubilized and counted. Results were expressed as the ratio of disintegrations per minute in the brain to disintegrations per minute in the perfusate (Rbr μ L/g) and are shown in Figure 8E. Each point on the plot represents the mean value from a cerebral hemisphere, with 4 rats in each treatment group. SEM bars represent experimental variability, and units are the ratio of disintegrations per minute in the brain to disintegrations per minute in the perfusate (Rbr μ L/g).

As shown in Figure 8E, perfusion with 10 nM amitriptyline for twenty minutes resulted in an increase in the cerebral penetration of the P-glycoprotein substrate [3H]-verapamil, indicating a decrease in P-glycoprotein transport activity. Thus, luminal amitriptyline exposure increased the brain deposition of a therapeutic P-glycoprotein substrate *in vivo*.

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CLAIMS

1. A method for delivery of an agent across the blood brain barrier of a subject comprising co-administering the agent with a LPA Receptor ligand, wherein the LPA Receptor ligand is administered in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.
5
2. A method of treating, preventing or ameliorating a condition, comprising administering to a subject a composition, wherein the composition comprises a therapeutic agent effective against the condition and an LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein thereby increasing delivery of the therapeutic agent across the blood brain barrier of the subject.
10
3. A method for modulating permeability of the blood brain barrier of a subject comprising administering to the subject a LPA Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.
15
4. The method of any one of the preceding claims, wherein the integrity or physical permeability of the blood brain barrier is not affected.
5. The method of any one of the preceding claims, wherein the LPA Receptor ligand is a LPA1 Receptor ligand, a LPA3 Receptor ligand, or both.
6. The method of any one of the preceding claims, wherein the LPA Receptor ligand is LPA or a structural analog of LPA.
20
7. The method of any one of the preceding claims, wherein the LPA Receptor ligand is selected from the group consisting of LPA, NAEPA, OMPT, oleoyl-thiophosphate, 2-oleoyl-LPA, and T13.
8. The method of any one of the preceding claims, wherein the LPA Receptor ligand is LPA.
25
9. The method of any one of the preceding claims, wherein the LPA Receptor ligand is an antidepressant.
10. The method of claim 9, wherein the LPA Receptor ligand is an antidepressant selected from the group consisting of fluoxetine, amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine.
30
11. The method of claim 9, wherein the LPA Receptor ligand is a tricyclic antidepressant.

12. The method of claim 9, wherein the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine.
- 5 13. The method of claim 9, wherein the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline and mianserin.
14. The method of claim 9, wherein the LPA Receptor ligand is amitriptyline.
15. The method of any one of the preceding claims, wherein the LPA Receptor ligand is a selective ligand of LPA1 Receptor.
- 10 16. The method of any one of claims 1, 2 and 4-15, wherein the agent is a therapeutic agent or a diagnostic agent.
17. The method of any one of claims 1, 2 and 4-16, wherein the agent is a therapeutic agent selected from the group consisting of an anti-bacterial agent, an anti-viral agent, an anti-mycotic agent, an anti-cancer agent, an analgesic, and a chemotherapeutic agent.
- 15 18. The method of any one of claims 1, 2 and 4-16, wherein the agent is a therapeutic agent and is a drug molecule.
19. The method of any one of claims 1, 2 and 4-16, wherein the agent is a diagnostic agent selected from the group consisting of an imaging molecule, and a labeling molecule.
- 20 20. The method of any one of claims 1, 2 and 4-19, wherein the agent and the LPA Receptor ligand are administered concurrently.
21. The method of any one of claims 1, 2 and 4-19, wherein the agent and the LPA Receptor ligand are administered sequentially.
- 25 22. The method of any one of claims 1, 2 and 4-21, wherein the route of administration of the agent or the LPA Receptor ligand or both is selected from the group consisting of intravenous, subcutaneous, transdermal, intramuscular, topical, and oral.
- 30 23. The method of any one of claims 1, 2 and 4-22, wherein delivery of the agent across the blood-brain barrier is increased about two-fold to about ten-fold compared to delivery of the agent in the absence of the LPA1 Receptor ligand.

24. The method of any one of the preceding claims, wherein the amount of the LPA Receptor ligand is sufficient to achieve a blood concentration level of about 0.1 to 1000.0 nM.
25. The method of any one of the preceding claims, wherein the subject is a human.
- 5 26. The method of any one of claims 2 and 4-25, wherein the condition is selected from the group consisting of a cancer, a neurological disease, and pain.
27. The method of claim 26, wherein the condition is cancer selected from the group consisting of brain cancer, neuroma, glioma, lymphoma, and glioblastoma.
- 10 28. The method of claim 26, wherein the condition is a neurological disease selected from the group consisting of amyotrophic lateral sclerosis (ALS), brain tumor, brain metastasis, schizophrenia, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, a disease associated with malfunction of the BBB, dementia, and HIV-associated dementia.
- 15 29. The method of claim 26, wherein the condition is pain selected from the group consisting of centrally mediated pain, peripherally mediated pain, neuropathic pain, acute pain, and chronic pain.
30. A pharmaceutical composition comprising a LPA Receptor ligand, an agent having therapeutic or diagnostic activity and a pharmaceutically acceptable excipient.
- 20 31. The pharmaceutical composition of claim 30, wherein the composition is formulated as a solid dosage form.
32. The pharmaceutical composition of claim 30, wherein the composition is formulated as a liquid dosage form.
33. The pharmaceutical composition of any one of claims 30-32, wherein the LPA Receptor ligand is a LPA1 Receptor ligand, a LPA3 Receptor ligand, or both.
- 25 34. The pharmaceutical composition of any one of claims 30-33, wherein the LPA Receptor ligand is LPA or a structural analog of LPA.
35. The pharmaceutical composition of any one of claims 30-33, wherein the LPA Receptor ligand is selected from the group consisting of LPA, NAEPA, OMPT, oleoyl-thiophosphate, 2-oleoyl-LPA, and T13.
- 30 36. The pharmaceutical composition of any one of claims 30-33, wherein the LPA Receptor ligand is LPA.
37. The pharmaceutical composition of any one of claims 30-33, wherein the LPA Receptor ligand is an antidepressant.

38. The pharmaceutical composition of claim 37, wherein the LPA Receptor ligand is an antidepressant selected from the group consisting of fluoxetine, amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine.
39. The pharmaceutical composition of claim 37, wherein the LPA Receptor ligand is a tricyclic antidepressant.
40. The pharmaceutical composition of claim 37, wherein the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline, mianserin, butriptyline, protriptyline, maprotiline, trimipramine, desipramine, doxepin, imipramine, amoxapine, and clomipramine.
41. The pharmaceutical composition of claim 37, wherein the LPA Receptor ligand is a tricyclic antidepressant selected from the group consisting of amitriptyline, nortriptyline and mianserin.
42. The pharmaceutical composition of claim 37, wherein the LPA Receptor ligand is amitriptyline.
43. The pharmaceutical composition of any one of claims 30-33, wherein the LPA Receptor ligand is a selective ligand of LPA1 Receptor.
44. The pharmaceutical composition of any one of claims 30-43, wherein the agent is a therapeutic agent or a diagnostic agent.
45. The pharmaceutical composition of claim 44, wherein the agent is a therapeutic agent selected from the group consisting of an anti-bacterial agent, an anti-viral agent, an anti-mycotic agent, an anti-cancer agent, an analgesic, and a chemotherapeutic agent.
46. The pharmaceutical composition of claim 44, wherein the agent is a therapeutic agent and is a drug molecule.
47. The pharmaceutical composition of claim 44, wherein the agent is a diagnostic agent selected from the group consisting of an imaging molecule, and a labeling molecule.
48. A method for delivery of an agent across a P-glycoprotein expressing barrier of a tissue or an organ of a subject comprising co-administering the agent with a LPA Receptor ligand, wherein the LPA Receptor ligand is administered in an amount sufficient to reversibly inhibit transport activity of P glycoprotein.

49. A method of treating, preventing or ameliorating a condition in a subject,
comprising administering to a subject a composition, wherein the composition
comprises a therapeutic agent effective against the condition and an LPA Receptor
5 ligand in an amount sufficient to reversibly inhibit transport activity of P
glycoprotein thereby increasing delivery of the therapeutic agent across a P-
glycoprotein expressing barrier of a tissue or an organ of the subject.
50. A method for modulating permeability of a P-glycoprotein expressing barrier of a
tissue or an organ of a subject comprising administering to the subject a LPA
10 Receptor ligand in an amount sufficient to reversibly inhibit transport activity of P
glycoprotein.
51. The method of claims 48-50, wherein the tissue or organ is selected from brain,
kidney, liver, intestine, retina, placenta and testes.
52. The method of claims 48-51, wherein the a P-glycoprotein expressing barrier is
15 blood brain barrier.

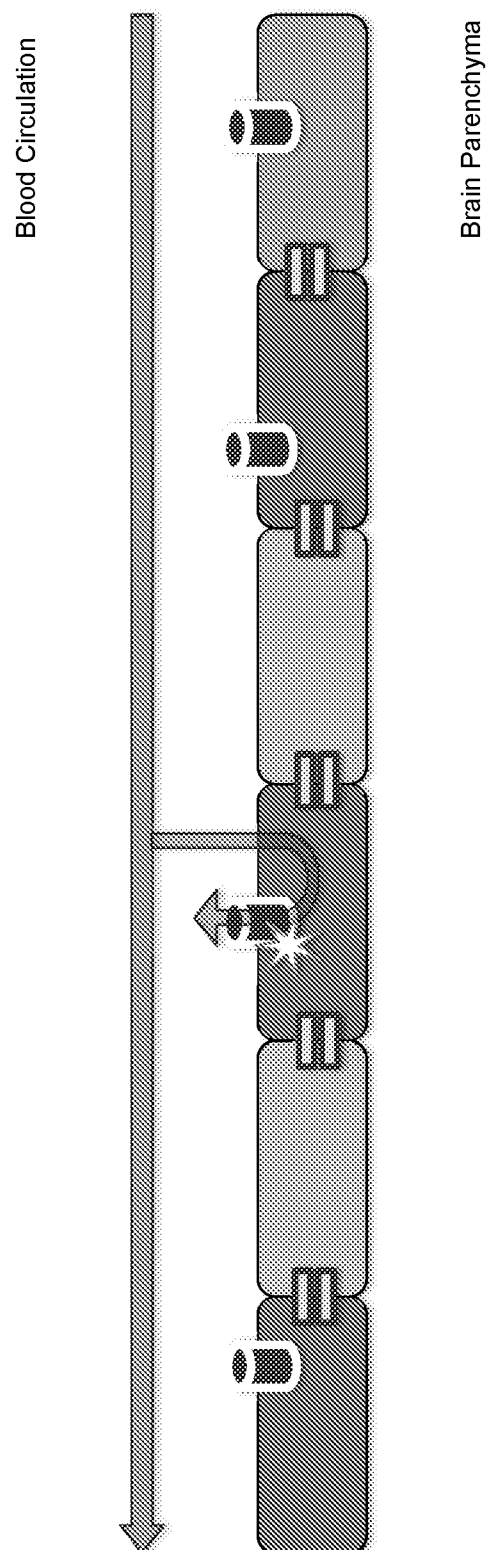
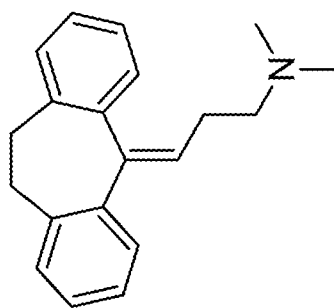
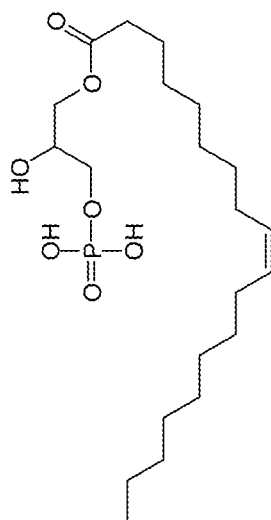


Figure 1



Tricyclic antidepressants (TCAs)



Lysophosphatidic acid (LPA)

Figure 2

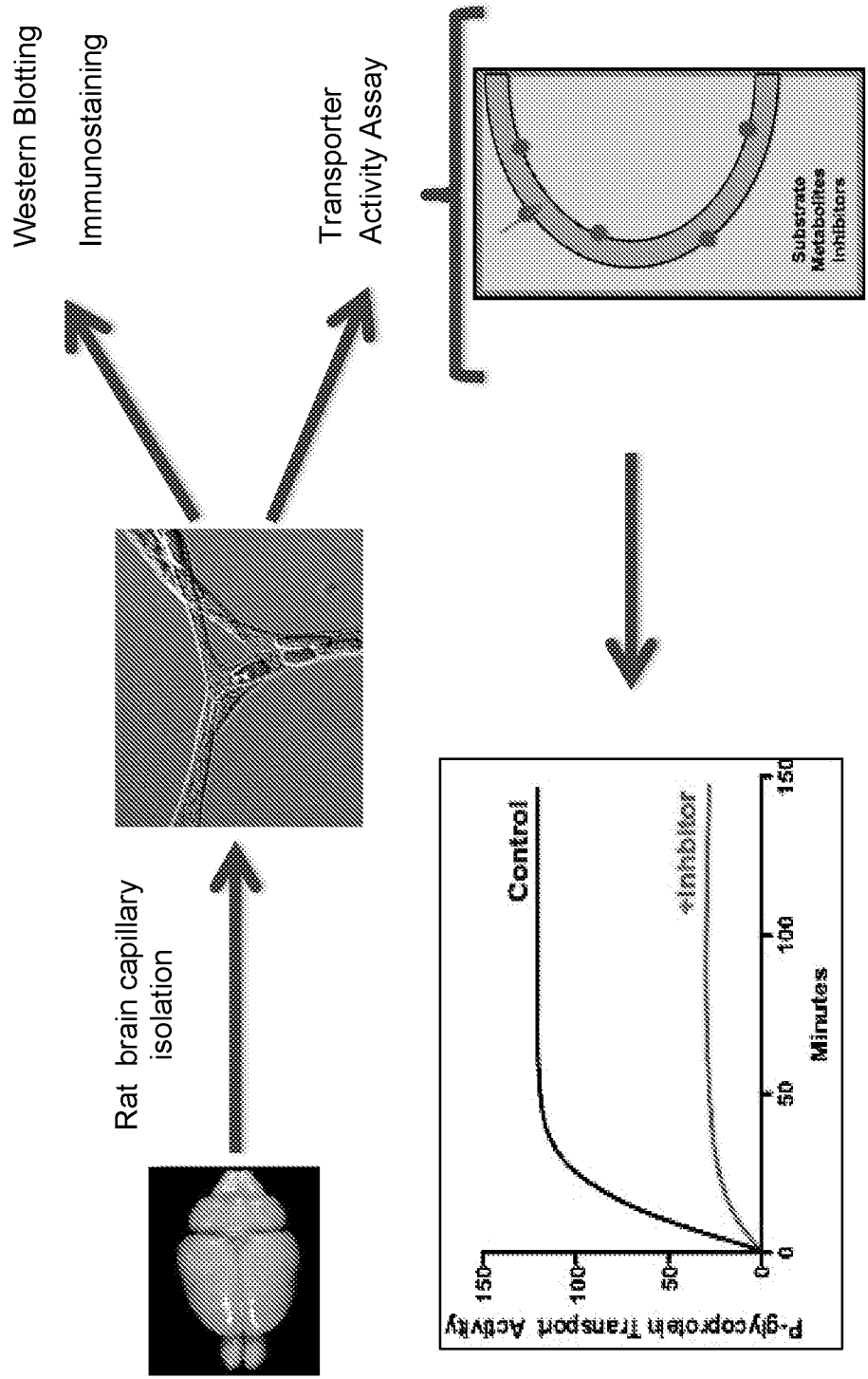


Figure 3

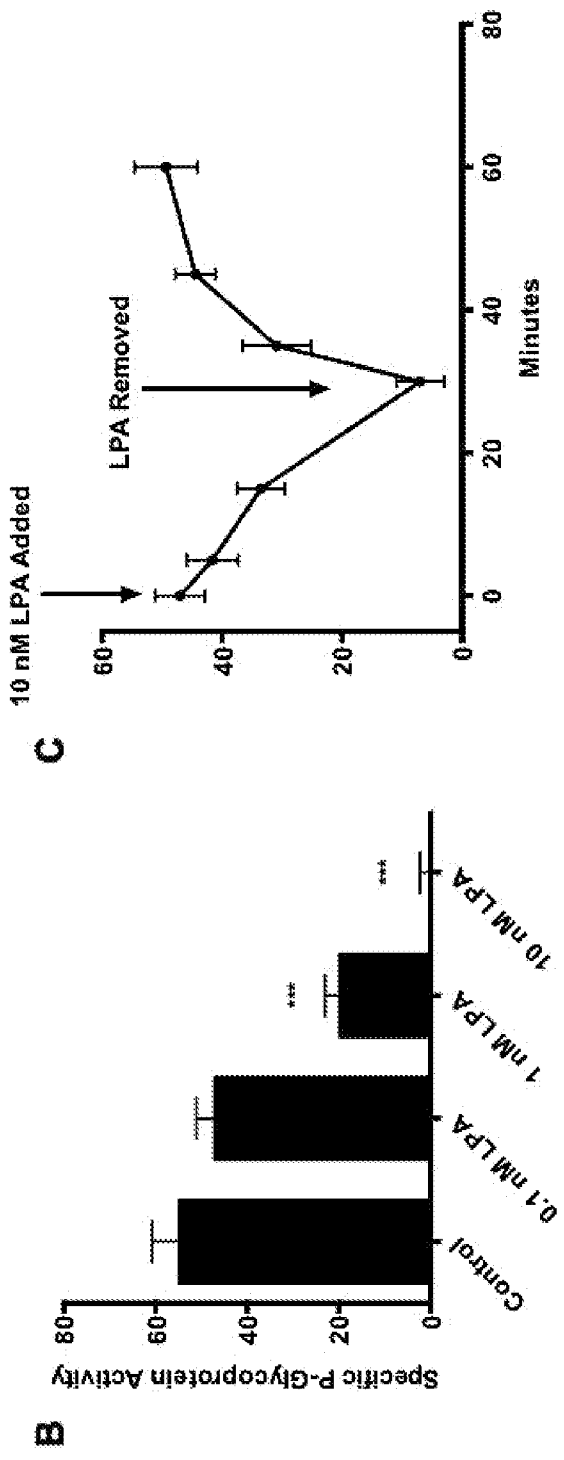


Figure 4

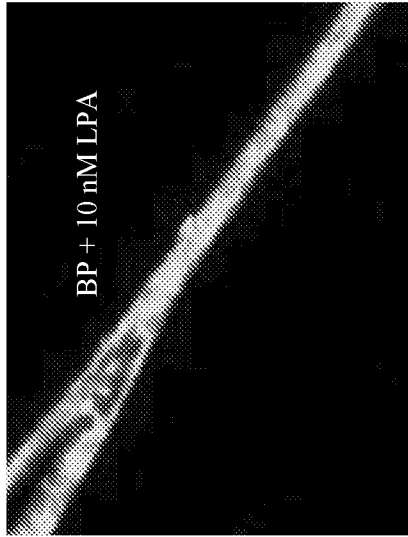
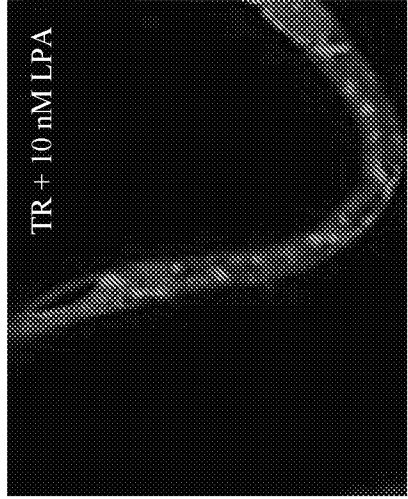
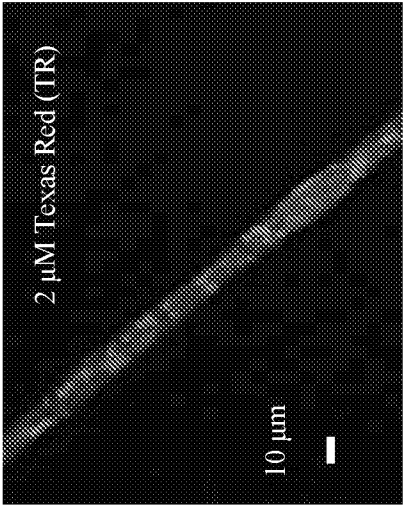
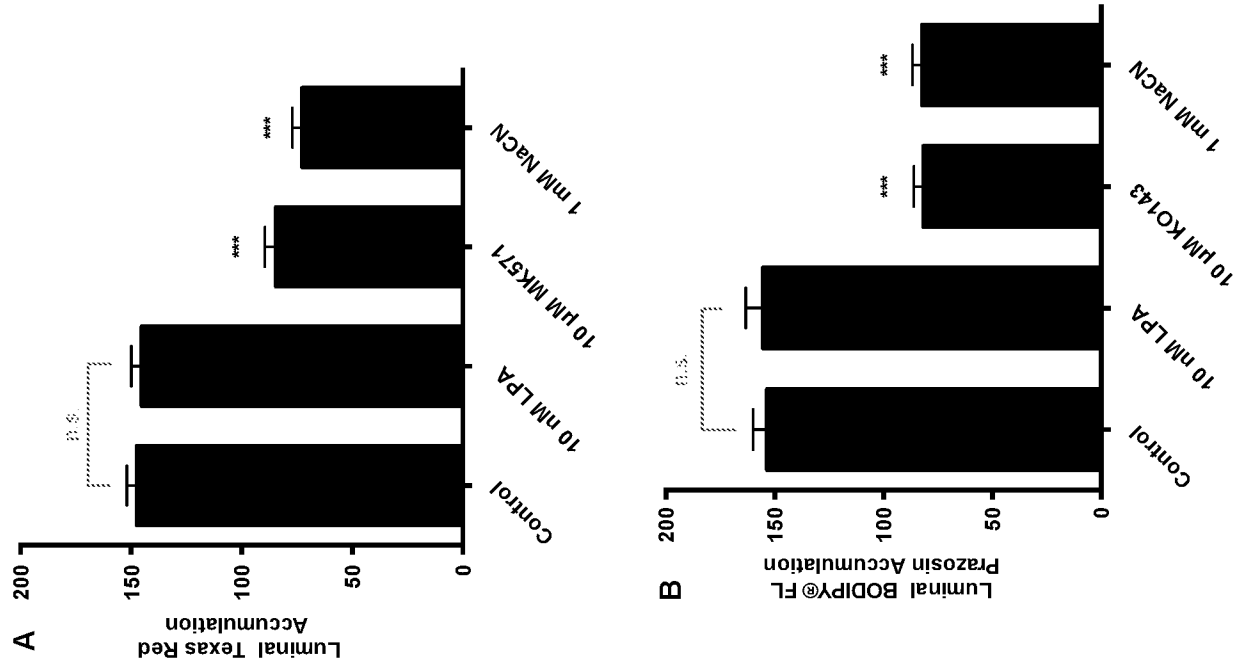


Figure 5

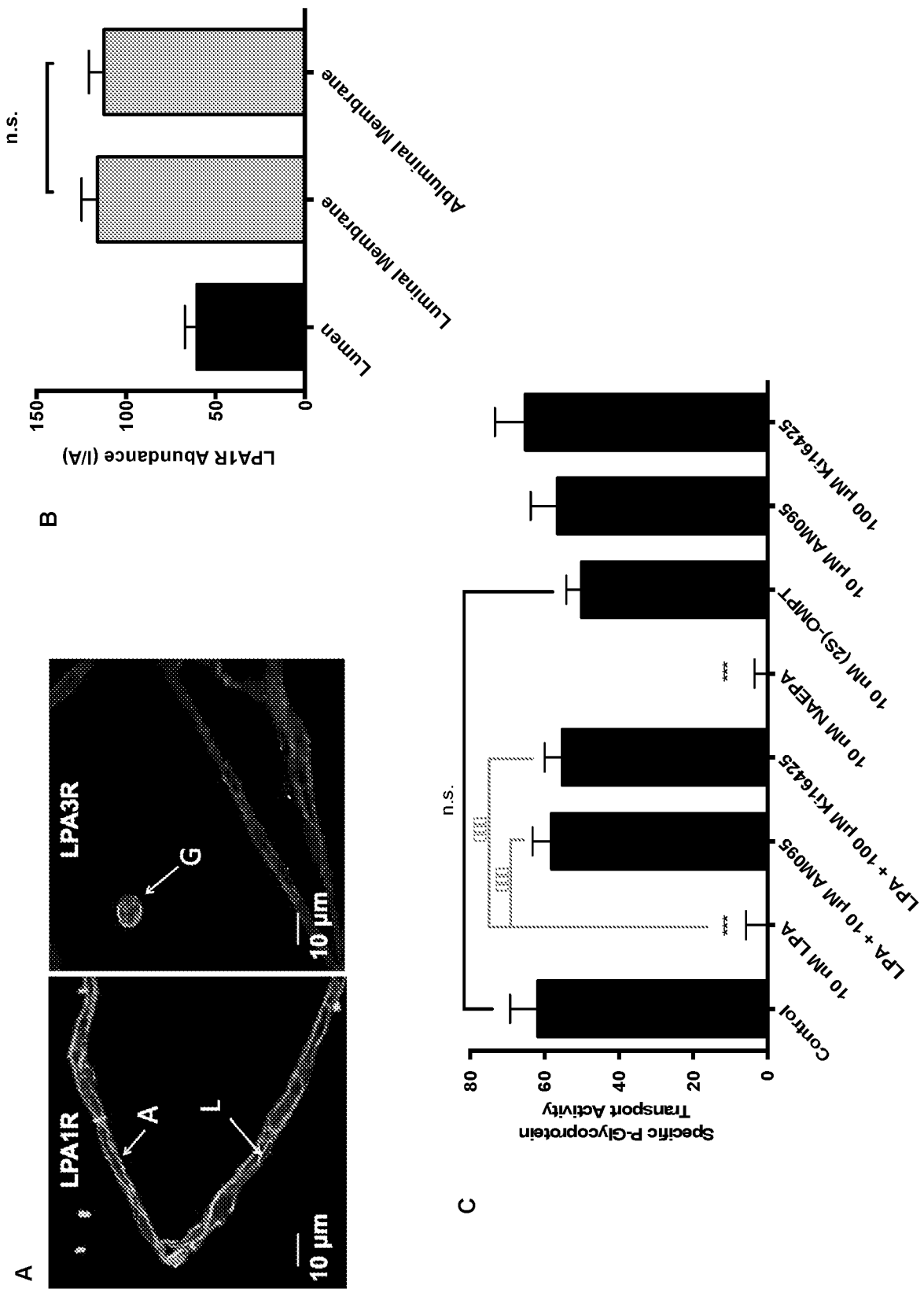
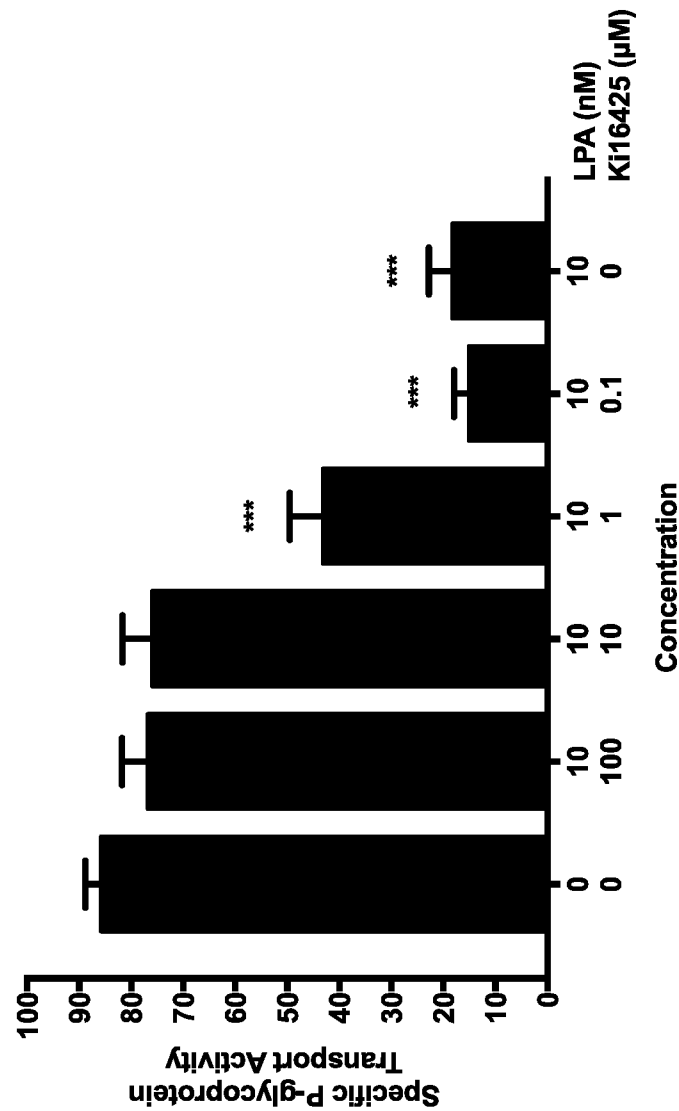


Figure 6

D



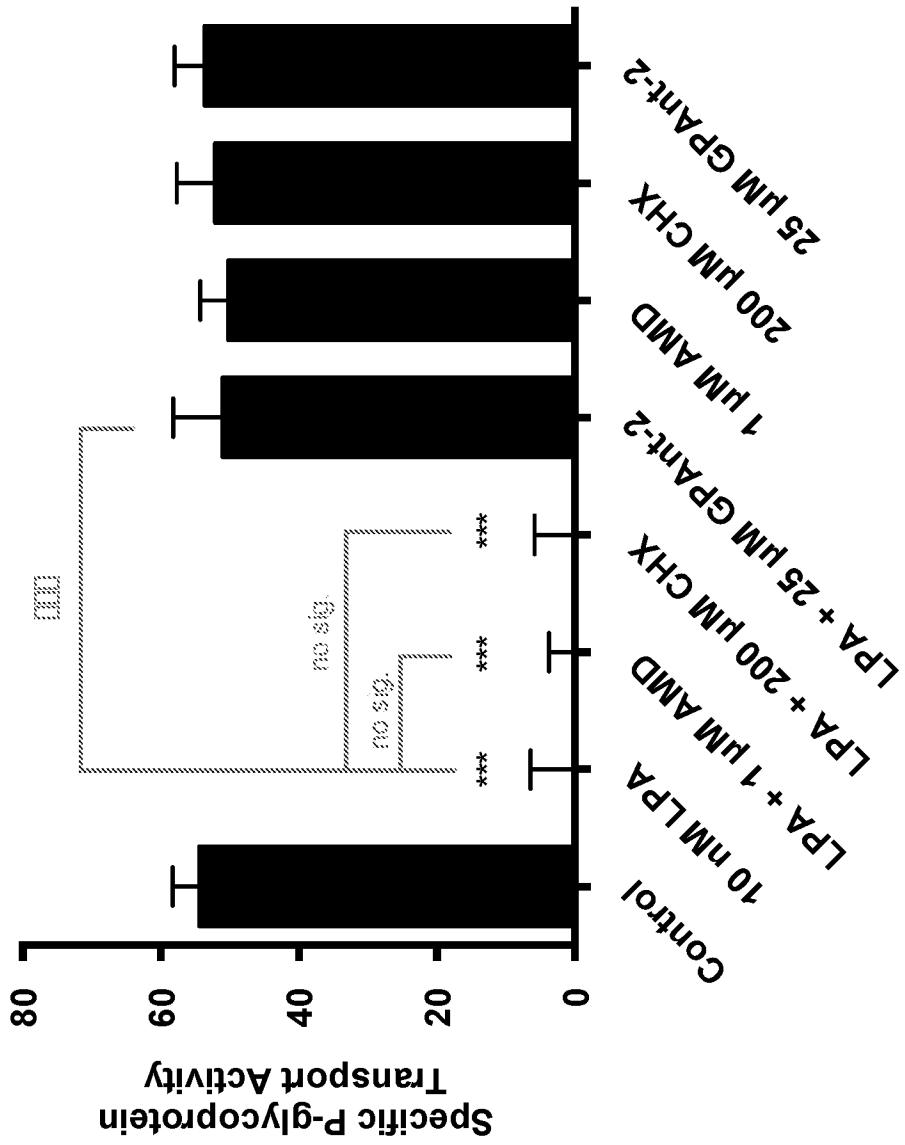


Figure 7

A

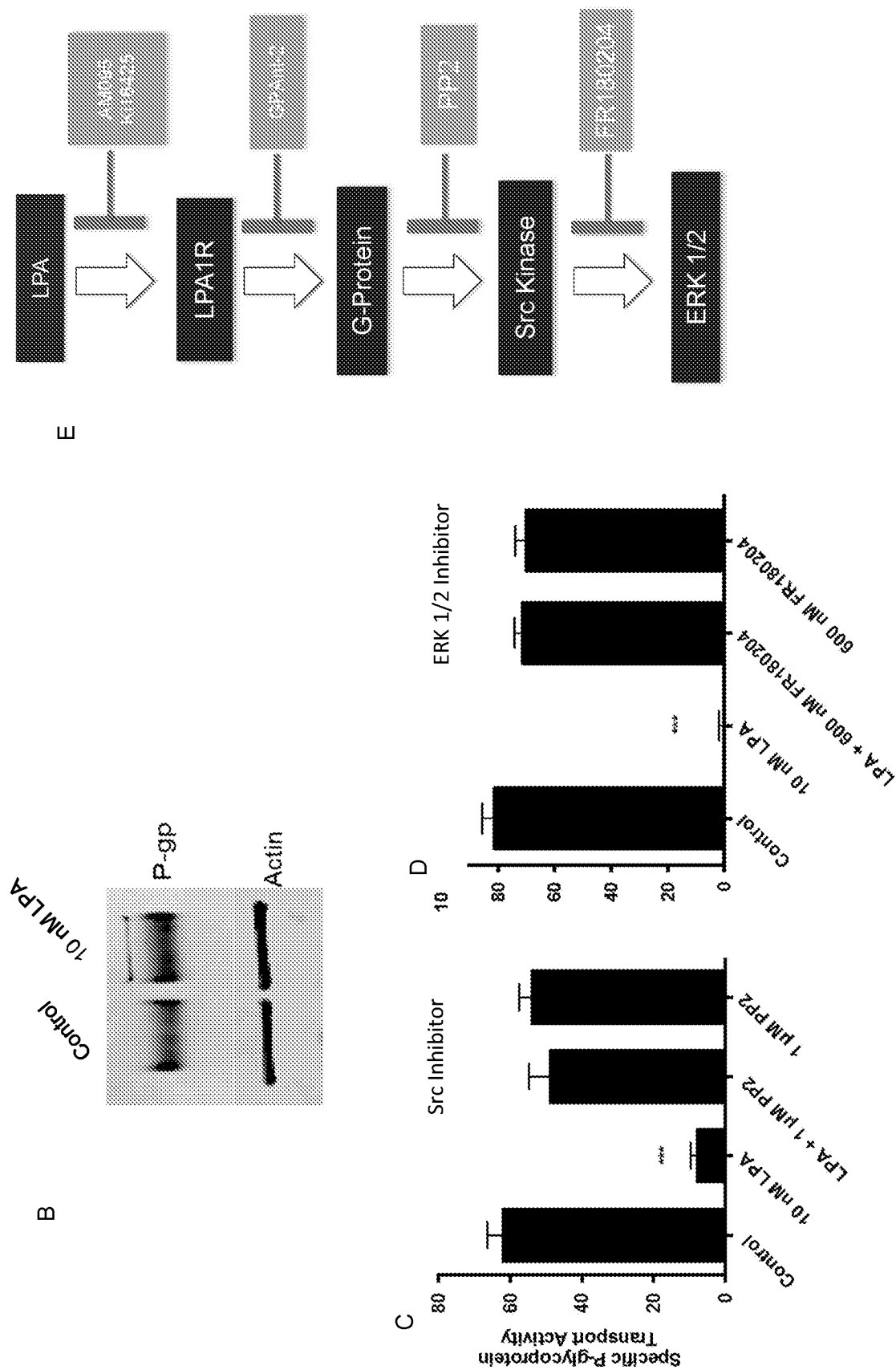


Figure 7 - Continued

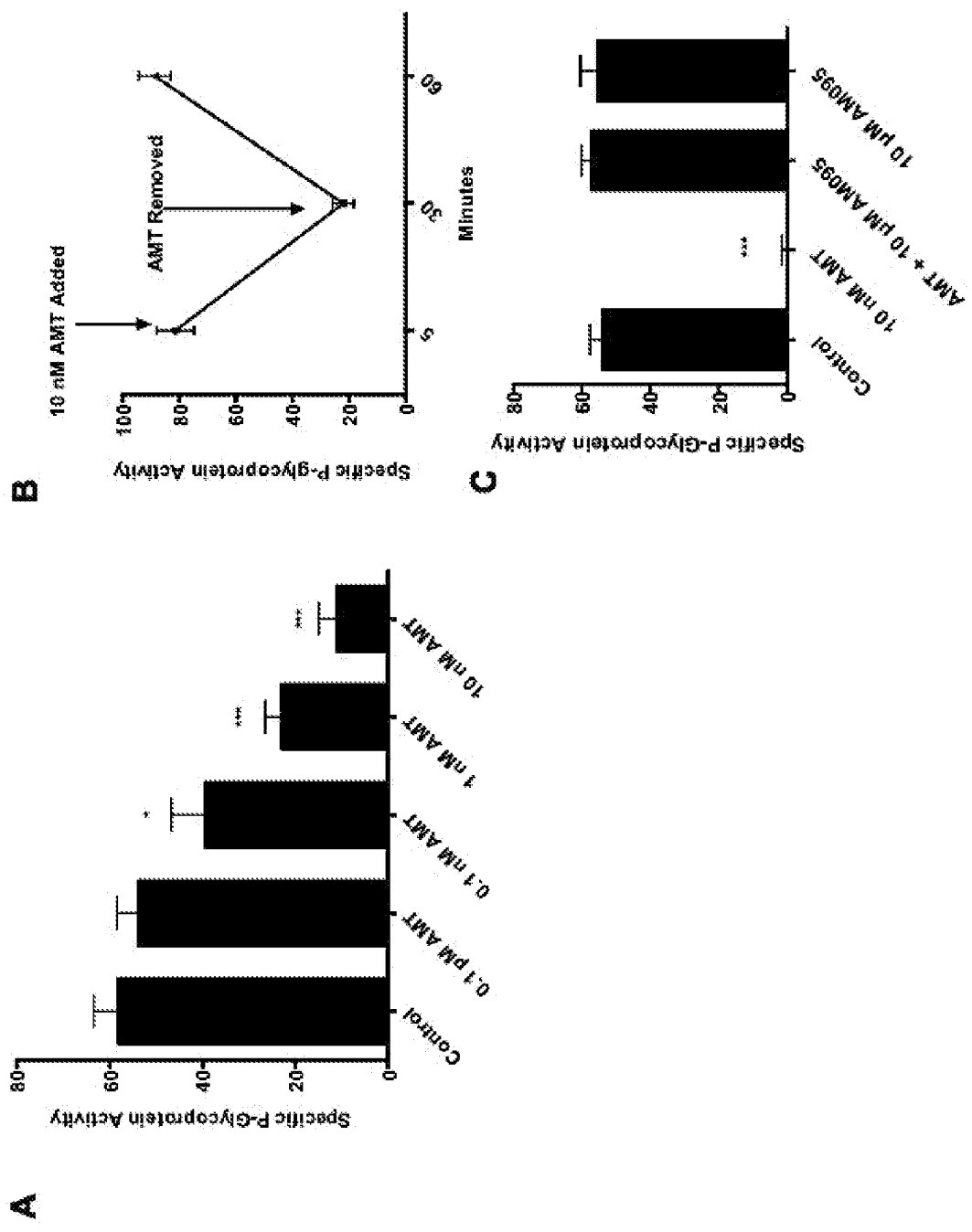
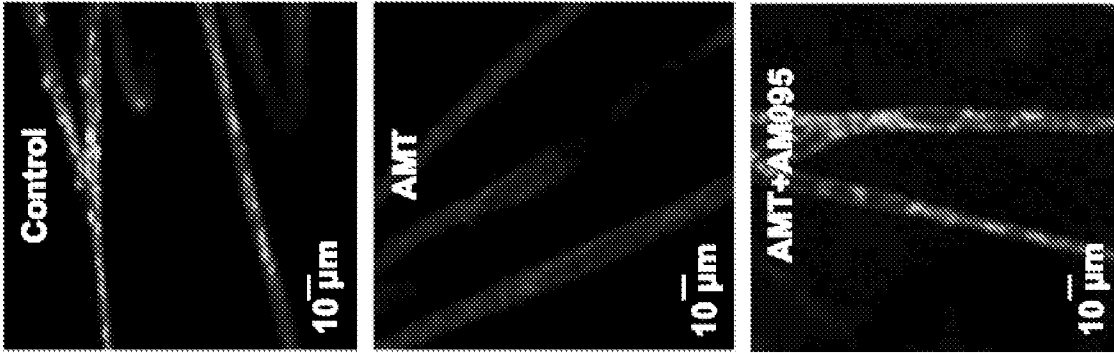


Figure 8



E

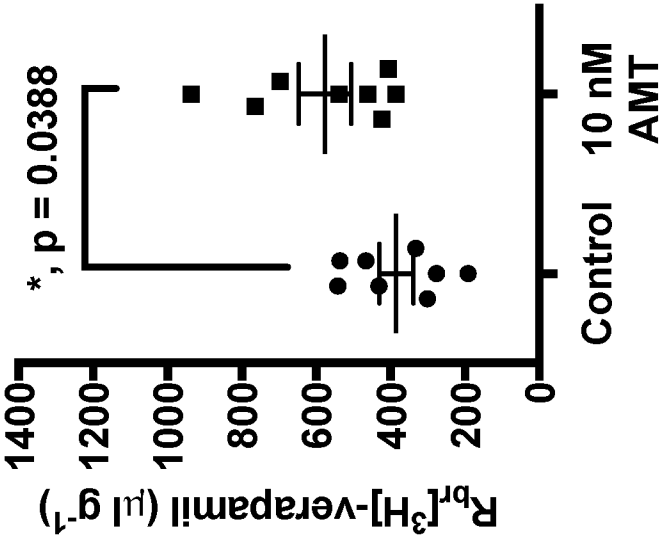


Figure 8 - Continued

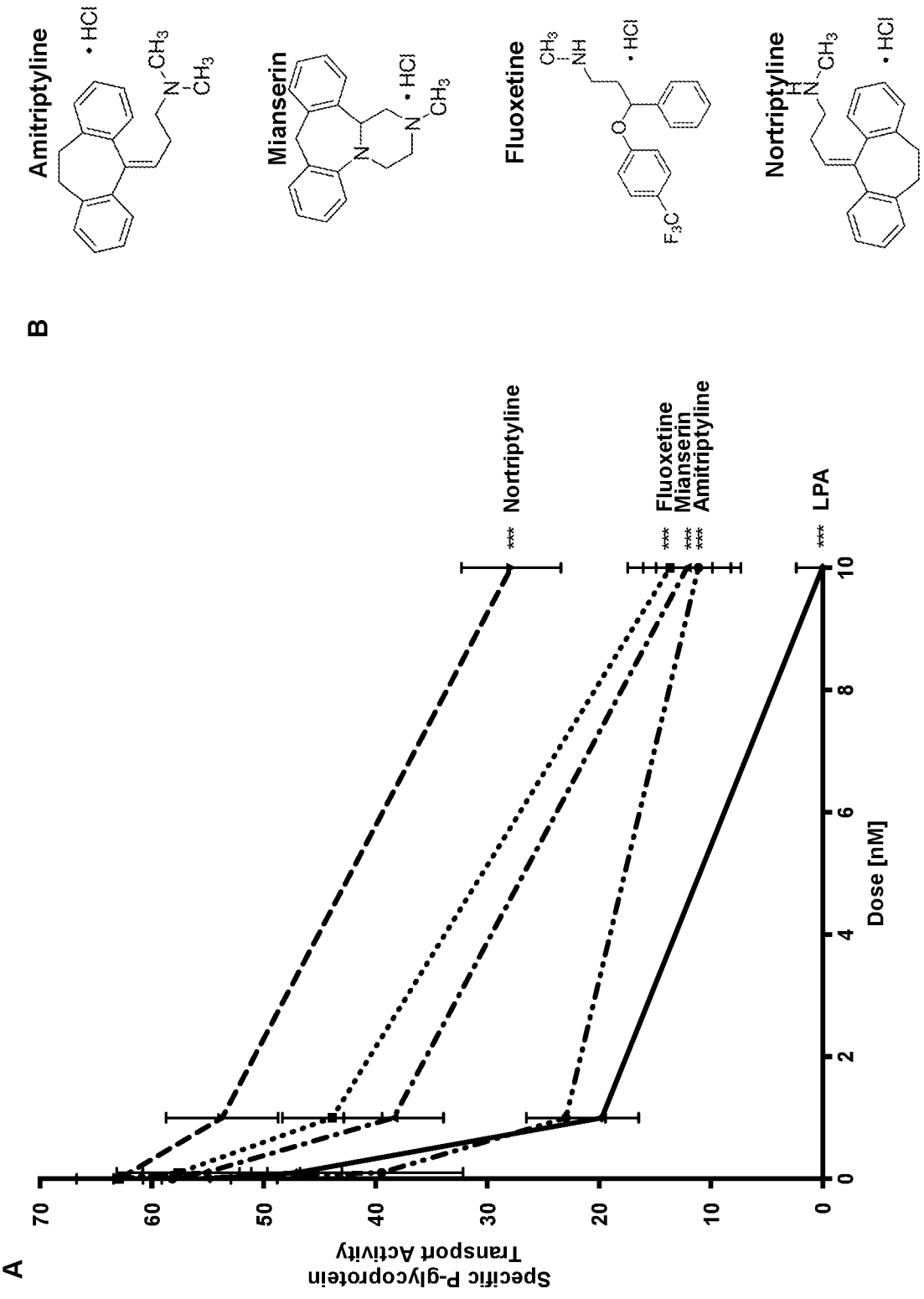


Figure 9

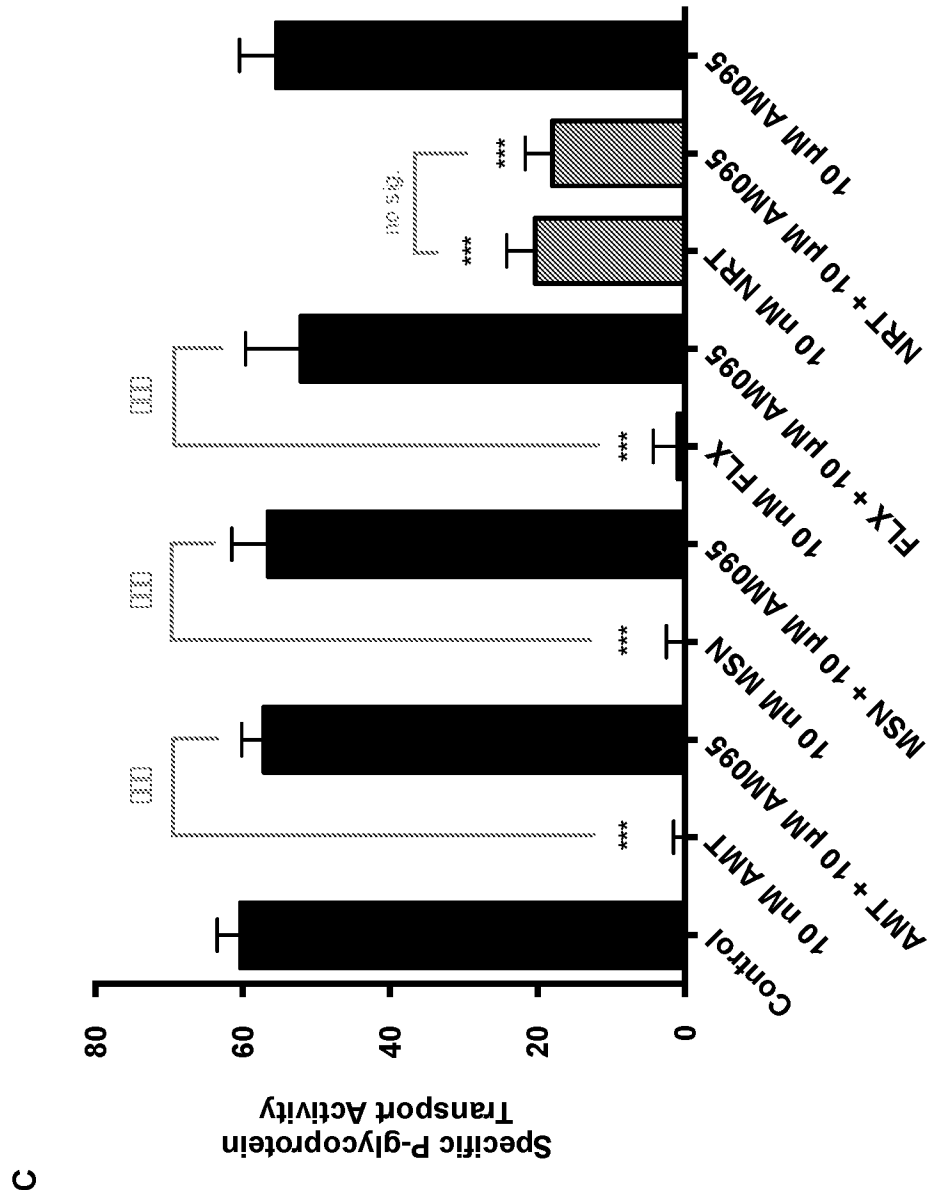


Figure 9 – Continued

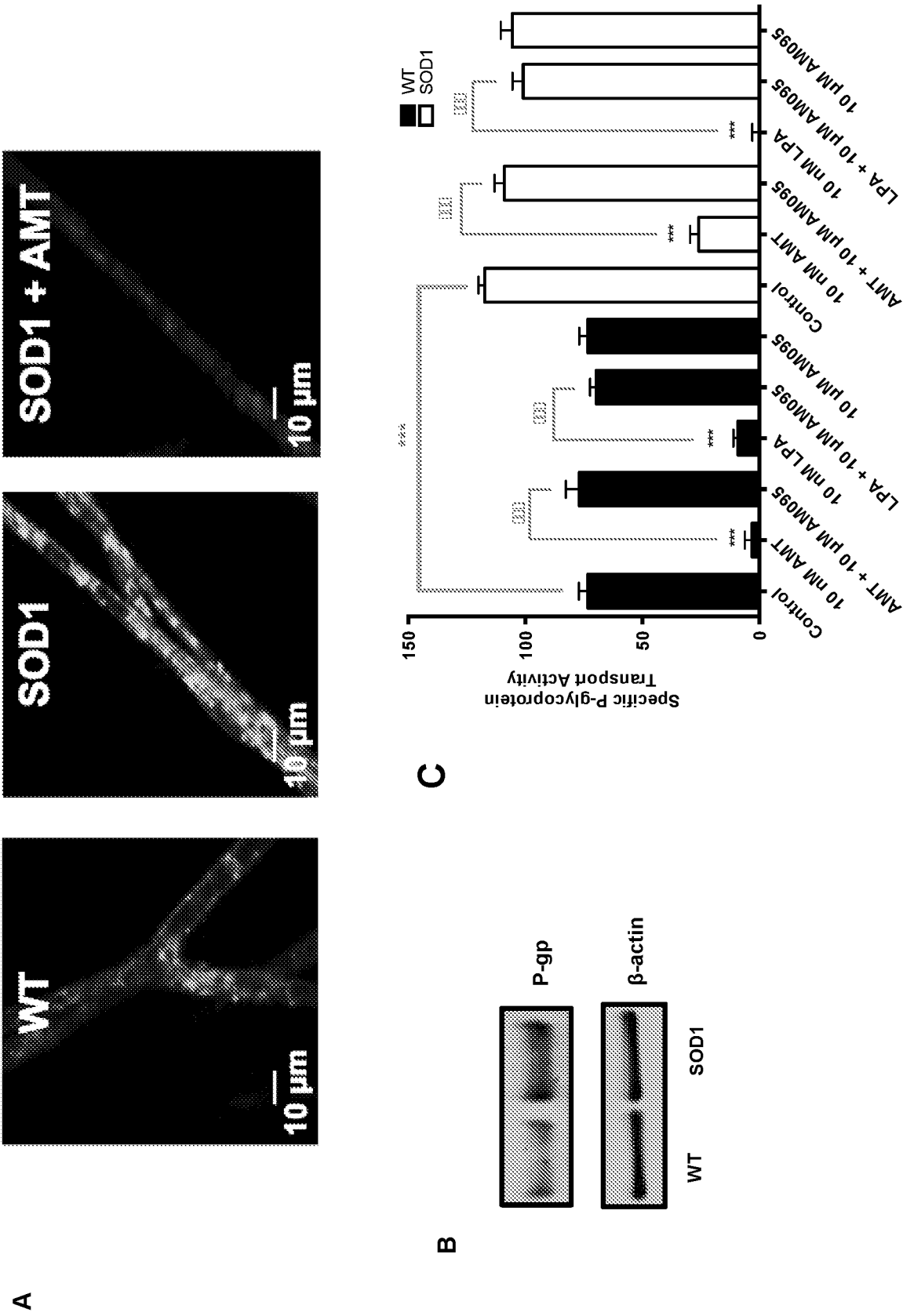


Figure 10

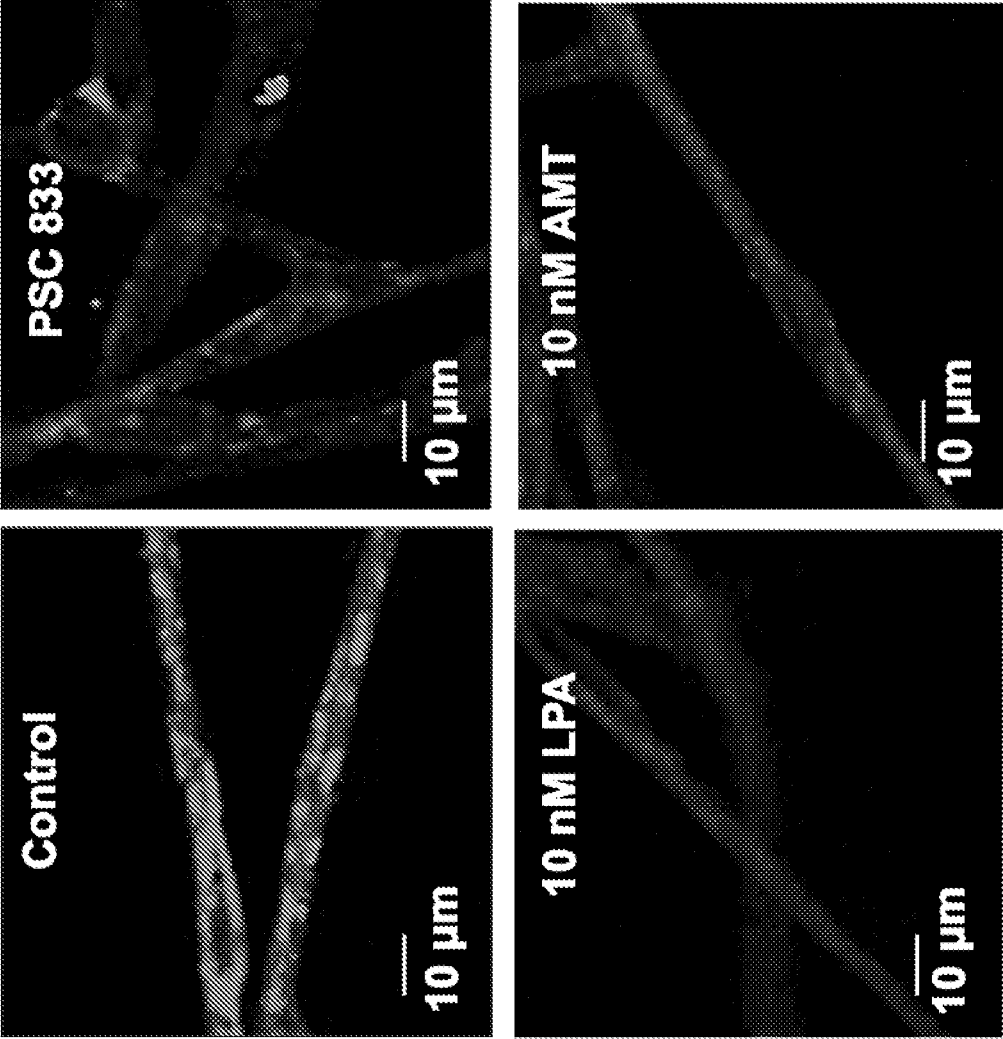


Figure 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/031206

A. CLASSIFICATION OF SUBJECT MATTER				
INV.	A61K31/135	A61K31/138	A61K31/277	A61K31/55
	A61K31/665	A61K45/06	A61P25/04	A61P25/08
	A61P25/18	A61P25/28	A61P35/00	A61P35/04
				A61P43/00
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) A61K				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	WO 2014/018932 A2 (BUNT ANTONIUS MARTINUS GUSTAVE [US]) 30 January 2014 (2014-01-30)	1-5, 9-33, 37-52		
A	the whole document, in particular par.002,032-034,038,042,055,056,060-063,07 8,099,0103,0104 and claims 26-31 ----- -/-	6-8, 34-36		
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.				
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family				
Date of the actual completion of the international search 24 July 2017		Date of mailing of the international search report 02/08/2017		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Albrecht, Silke		

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/031206

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUN-SHENG WANG ET AL: "Population pharmacokinetic analysis of drug-drug interactions among risperidone, bupropion, and sertraline in CF1 mice", PSYCHOPHARMACOLOGY, SPRINGER, BERLIN, DE, vol. 183, no. 4, January 2006 (2006-01), pages 490-499, XP019326735, ISSN: 1432-2072, DOI: 10.1007/S00213-005-0209-Y	1-5,9, 16-33, 37,44-52
A	abstract table 3 page 496, column 2, paragraph 1 page 497, column 1, paragraph 3 - column 2, paragraph 2	6-8, 10-15, 34-36, 38-43
X	----- J. WEISS ET AL: "Inhibition of P-Glycoprotein by Newer Antidepressants", JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 305, no. 1, 1 April 2003 (2003-04-01), pages 197-204, XP055393237, US	1-5,9, 10, 16-33, 37,38, 44-52
A	ISSN: 0022-3565, DOI: 10.1124/jpet.102.046532 abstract table 2 -----	6-8, 11-15, 34-36, 39-43

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/031206

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014018932 A2	30-01-2014	AU 2013295549 A1	19-03-2015
		CA 2880236 A1	30-01-2014
		CN 104736144 A	24-06-2015
		EP 2877159 A2	03-06-2015
		HK 1210957 A1	13-05-2016
		JP 2015523400 A	13-08-2015
		US 2014235631 A1	21-08-2014
		WO 2014018932 A2	30-01-2014
