Aleph Pharmaceuticals

Universidad Andrés Bello

Developing a novel class of antidepressants targeting astrocytes

Dr. Jimmy Stehberg

Sept, 2019

EXECUTIVE SUMMARY

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Technology	Novel small molecule antidepressants targeting astrocytes
Novel cell target	First known compounds to target brain astrocytes, a cell type that participates in neuronal synapse signaling
Validated mechanism of action	By targeting Cx43 hemichannel-mediated astroglial release of D-serine and glutamate, the activity of NMDA receptors is diminished, inducing antidepressant effects like KETAMINE, but without its sedative effects.
Lead candidate with fast antidepressant effects in rodents	Lead compound with rapid (<10 min) antidepressant effects when administered systemically in rats
Issued intellectual property	Patent granted (WO2013179264A) in US. Additional follow-on compounds in drafting.
Financing to-date	$600K Competitive Grants. $50K private investment.
Team	Expertise in drug discovery, *in vitro* and *in vivo* assays

PROBLEMS WITH CURRENT ANTIDEPRESSANTS

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- 50% of people with depression do not respond to current antidepressants.
- Current generic and branded antidepressants take 3 weeks to exert their effects, often requiring the prescription of other drugs.
- All drugs in the market target neurons but there are no drugs targeting other brain cells.
- Ketamine has demonstrated rapid antidepressant effects, but side effects and abuse potential limit broad utility

KETAMINE: VALIDATED ANTIDEPRESSANT ACTIVITY VIA NMDAR

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MDD patients show abnormalities in NMDAR-mediated glutamate transmission

Beneyto et al., 2007; Beneyto and Meador-Woodruff, 2008; Feyissa et al., 2009; Karolewicz et al., 2009, 2005.

Chronic antidepressant treatment and electroconvulsive stimulation change NMDAR-binding properties

No[illegible]et al., 1993; Paul et al., 1994, 1993

A[illegible]epressants alter region-specific expression of NMDARs

Nowak et al., 1996, 1993; Skolnick, 1999; Skolnick et al., 1996

Placebo-controlled studies have demonstrated that intravenous (IV) administration of s[illegible]esthetic doses (0.5 or 1 mg/kg over 40 min) of ketamine induces rapid (within hours) antidepressant effects that last up to 7 days

Bobo et al., 2016; Fond et al., 2014; Fava et al., 2018

Ketamine is effective in drug-resistant patients who have tried two or more typical a[illegible]epressants

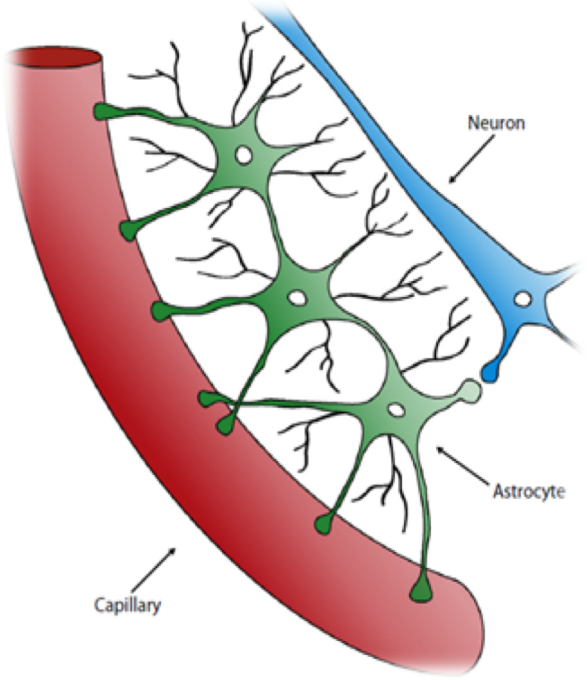
Diaz-Granados et al., 2010a, 2010b; Ibrahim et al., 2011; Kranaster et al., 2011; Phelps et al., 2009; Zarate et al., 2006

Single dose and repeated doses of Ketamine have been shown to be effective in several m[illegible]analyses

Fond et al., 2014; Bartoli et al., 2017

DOWNSIDE: KETAMINE IS ADDICTIVE, USED AS A RECREATIONAL DRUG; INDUCES HALLUCINATIONS; IT IS EASY TO OVERDOSE SO IT HAS TO BE ADMINISTERED BY A DOCTOR.

ASTROCYTES: A NOVEL TARGET FOR ANTIDEPRESSANTS



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eview Paper

ole of astrocytes in memory and psychiatric disorders

Moraga-Amaro[a], J.M. Jerez-Baraona[a], F. Simon[b], J. Stehberg[a,*]

- Astrocytes release neurotransmitters into synapses that activate neurons.
- Decreased release from astrocytes results in reduced neuron-to-neuron synaptic activity.
- Aleph Pharma has demonstrated the ability to selectively reduce astroglial release of neurotransmitters resulting in a decrease in NMDAR activity and antidepressant effects

ASTROCYTES: A NOVEL TARGET FOR KETAMINE-LIKE ANTIDEPRESSANTS

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- **Ketamine** acts by inhibiting a glutamate receptor known as **NMDAR**
- **Astrocytes** release a transmitter (D-serine) required for **NMDAR** activity
- This release is via connexin 43 (Cx43) hemichannels, which are expressed only in astrocytes.
- Thus, inhibiting Cx43 hemichannels reduces the release of D-serine from astrocytes, **decreases NMDAR activity in neurons, inducing similar antidepressant effects as ketamine.**
- Ketamine can block completely NMDARs inducing **sedative effects** and an **overdose can lead to death.** Blocking astrocyte release via Cx43 hemichannels **decreases** NMDAR activity, but does block it completely.

To-date, we have demonstrated antidepressant activity in animals without sedative effects or death at clinically relevant doses.

ASTROGLIAL CX43 HEMICHANNELS AND DEPRESSION

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MDD patients and animal models for depression show an increase in Cx43 HC activity in depression relevant brain regions

Orellana et al., 2015; Miguel-Hidalgo et al., 2014

Antidepressants decrease Cx43 HC activity in astrocytes

Jeanson et al., 2016

Cx43 KO mice show a reduction of depression-related behaviors

Quesseveur et al., 2015.

BLOCKING ASTROGLIAL CX43 HEMICHANNELS DECREASES NMDAR ACTIVITY IN NEURONS AND HAS ANTIDEPRESSANT EFFECTS

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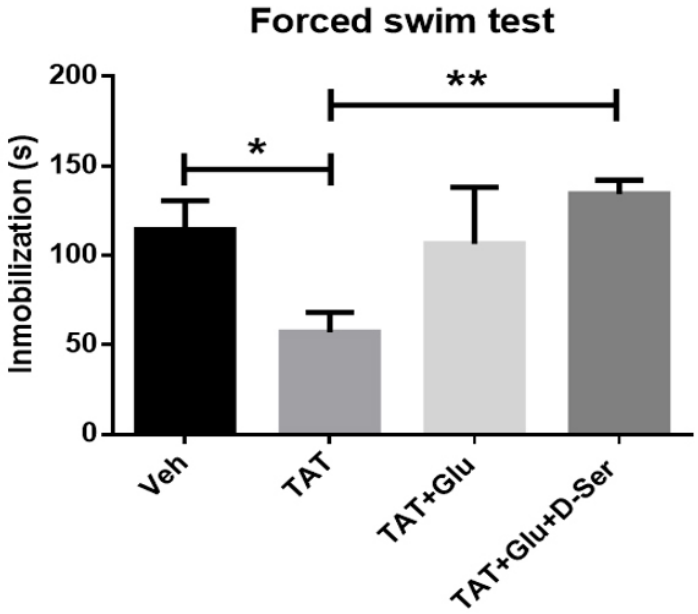
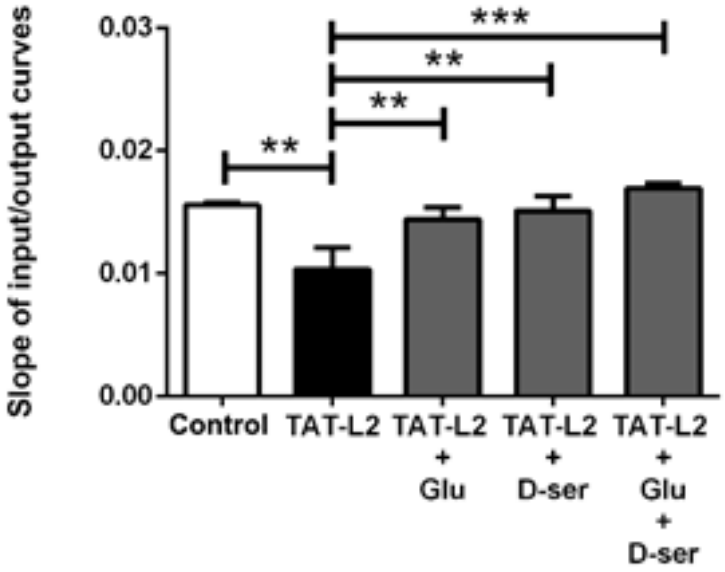
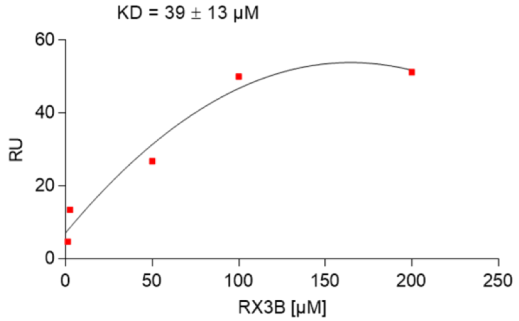




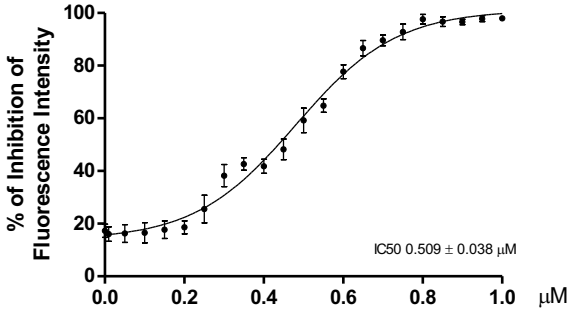
TAT-Cx43L2 (TAT-L2) is a mimetic peptide that blocks Cx43 hemichannels without affecting Cx43 gap junctions. (A) When injected into the hippocampus, TAT-L2 induces antidepressant effects (measured using the FST in rats that underwent chronic restraint stress), which can be prevented by co-infusion with glutamate and D-serine. (B) When hipocampal slices are incubated with TAT-Cx43L2, NMDAR activity is reduced, effect that can be prevented by addition of glutamate and D-serine. TAT-L2 has no effects on NMDAR activity in hipocampal neuronal cultures void of astrocytes.

Rx3B binds to the target protein and has effects *in vitro* at nM range



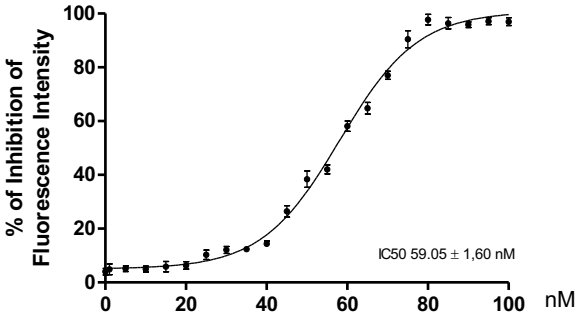
Interacts with the target protein with a KD of 30-60 µM

Measured using plasmon resonance



IC50 in transferected HeLa cells at nM range

Measured using dye uptake

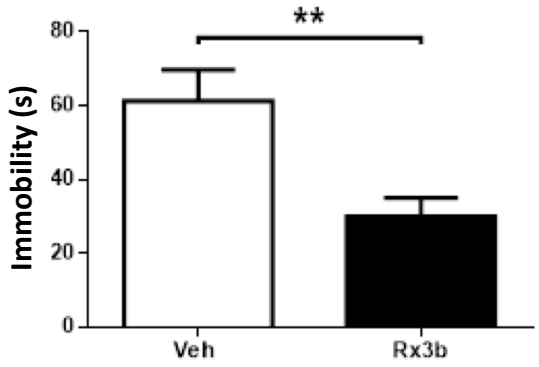


RX3B

IC50 in astrocytes ~50 nM

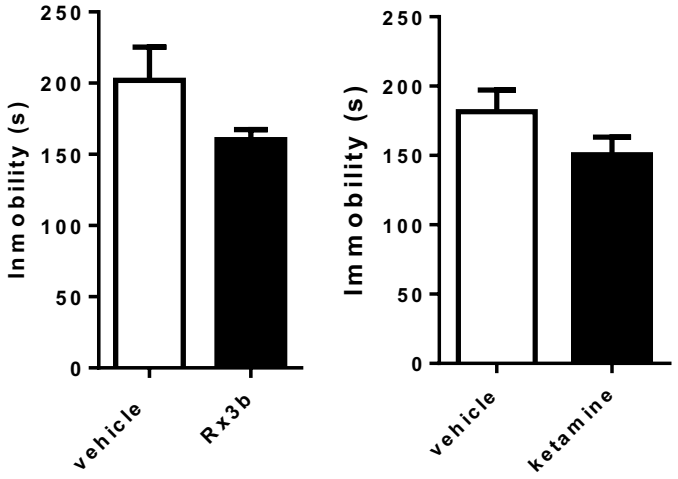
Measured using dye uptake

Antidepressant effects of Rx3B *in vivo*



Shows antidepressant-like effects when injected systemically (s.c) at a 13 µg/Kg dose .

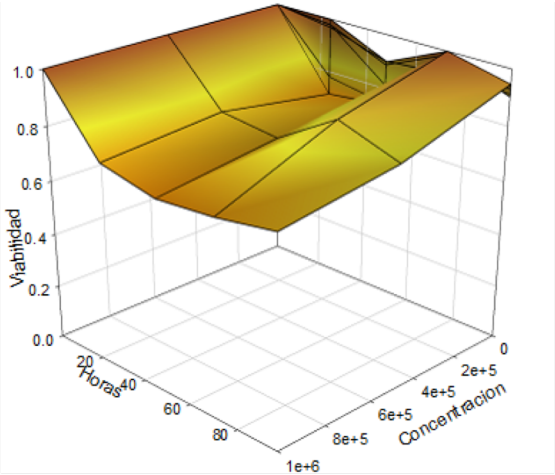
Measured in FST on animals that underwent chronic restraint stress.



Shows antidepressant effects similar to ketamine when injected systemically

Measured in TST on animals that underwent chronic restraint stress.

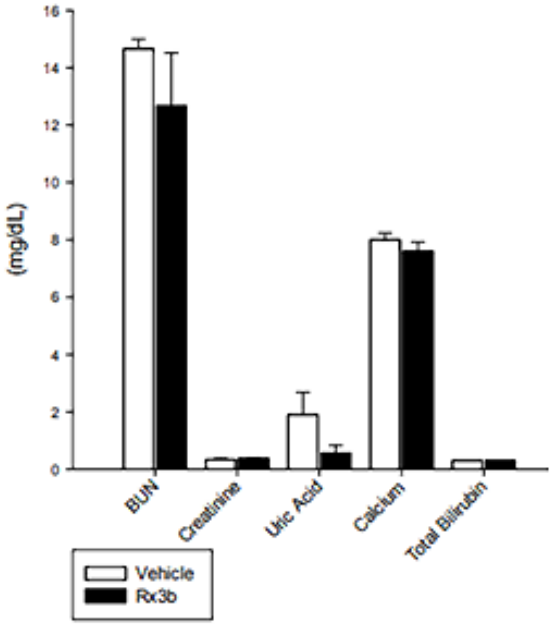
Side of effects of our lead compound

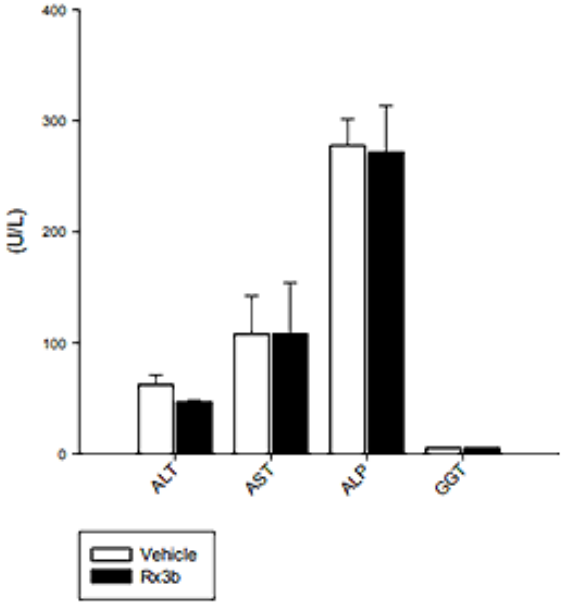


Low celular toxicity (viability assay using MTT of transfected HeLa cells)

No sedative effects. No reduction in locomotion in the open field. No noticeable behavioral abnormalities.

Low toxicity (LD50> 325 mg/kg), high oral absorption (95%)
Obtained module *QikProp* of the Suite Maestro of Schrödinger





No toxicity on organ function after single dose on analytes implicated in renal function (Blood uric nitrogen (BUN), Creatinin, Uric acid, Calcium) and hepatic function (total Billirubin; blood transaminases (ALT, AST, GGT) and phosphatases (ALP))
Obtained using Piccolo, blood biochemical assay, n=3.

COMPETITION

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DRUG	TIME TO ATTAIN EFFECTS	NOVELTY OF TARGET	SIDE EFFECTS
Serotonin transporter (SSRIs)	3-4 weeks	Not novel	Low
Cyclic antidepressants	3-4 weeks	Not novel	High
Monoamine oxidase inhibitors (MAOIs)	3-4 weeks	Not novel	High
Ketamine	<1 hour	Not novel	High*
Esketamine (Spravato)	<1 hour	Not novel	Medium*
RX3B	<1 hour	**Novel**	**Low**

* Requires treatment performed by a doctor

AN INTERNATIONAL SCIENTIFIC & BUSINESS TEAM

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Investigators:

- Dr. Jimmy Stehberg (*in vitro, in vivo* models).
- Dr. Felipe Simon (*in vitro* screening).
- Dr. Danilo González (*In sillico* work; small molecules).

Outside collaborators:

- Fraunhofer IME, Germany.
- UGhent, Belgium (Luc Leybaert).
- KULeuven, Belgium (Geert Butynck).
- UDD, Chile, (Mauricio Retamal).

Advisors:

- Nancy Levy
- Francisco Chiang
- Amanda Wagner

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Conclusion: Aleph Pharma is seeking a partner to finance IND-enabling toxicology and/or in-license our novel molecules

ADVANCES SINCE JUNE

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Goals & Advances

Goals set in june	Advance to date
Get funding for preclinical studies (ADME, safety target screening, oral availability, non-GLP)	We obtained funding for ADME and PK from the technology transfer office of UNAB. Goal was reached within the time set (July-sept)
Perform ADME and safety studies	We performed a UPLC so far. We are currently synthetizing sufficient molecule to perform the ADME/PK study. We hope to meet the deadline (december)
Set a partnership with a Pharmaceutical company	Has not begun yet. Amanda has helped us greatly by correcting the presentation deck. She has agreed to meet with people at Cadent and help setting up meetings. Hopefully we will accomplish this goal before or during our stay in Boston in december.

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Astrodepressants

Antidepressants Targeting Astrocytes

Dr. Jimmy Stehberg

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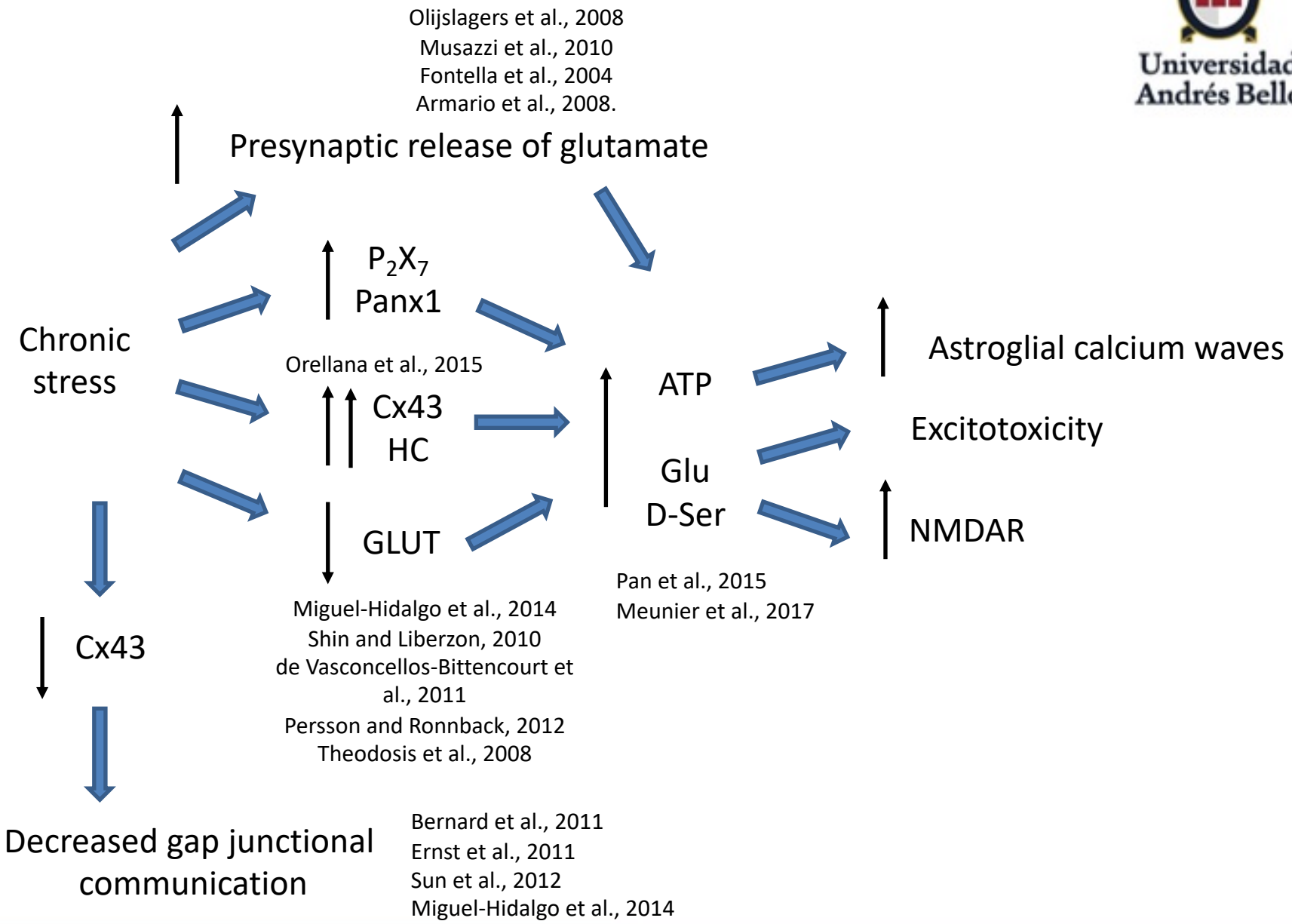
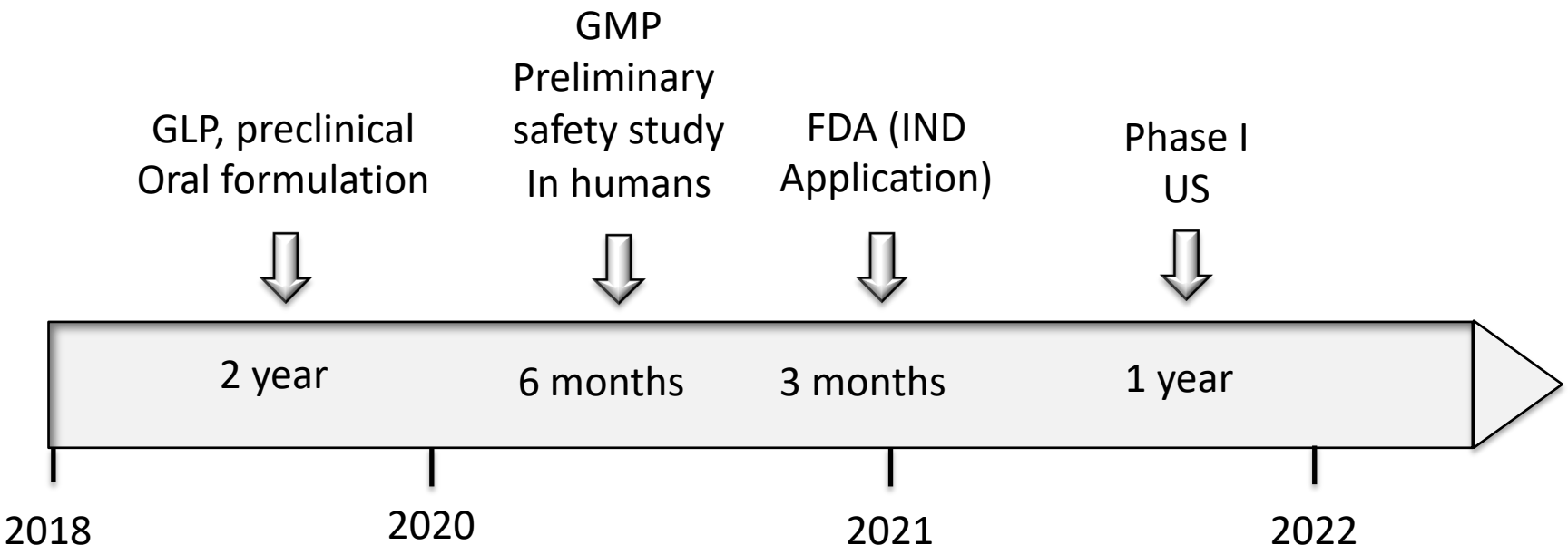
Annexes

What we know about chronic stress in the glutamatergic synapse



TIMELINE


GLP, preclinical
Oral formulation
GMP
Preliminary
safety study
In humans
FDA (IND
Application)
Phase I
US
2 year
6 months
3 months
1 year
2018
2020
2021
2022

TRACTION

- Funding: $700K in non-dilutive grants.
- We have published 7 papers on the role of our target in psychiatric disorders, including depression, anxiety and memory.
- We have setup active collaborations on this topic with several labs in Chile, Belgium, Germany and France.

*US patent granted on a small molecule for use in depression: US 14/404.358 "Use of compounds that selectively modulate astrocytic release of substances…"