Pronexus Analytical AB

Pronexus Analytical AB (Inc.) was founded in 2003 by Professors Urban Ungerstedt, Kjell Fuxe and Jan Kehr as a spin-off company linked to the Karolinska Institutet Science Park in Stockholm.

Mission statement

Pronexus Analytical AB is an independent preclinical contract research organization (CRO) offering advanced services and collaborative projects focused on PK/PD studies using microdialysis and neurochemical monitoring in rodent models of CNS and metabolic disorders. The bioanalytical services include UHPLC-MS/MS, standard HPLC techniques, enzyme assays and immunoassays.

Pronexus research laboratories with full regulatory approvals include the central bioanalytical laboratory and the animal research facility.

Pronexus expertise in CNS drug discovery

Preclinical phase 5 – 6 years

Protein targets: Enzyme Receptor Ion-channel Uptake inhibitor *Other targets:* Gene delivery	Cells expressing the target	HTS Hits	Lead optimization *in vitro* Medicinal chemistry Organic chemistry ADME Absorption Distribution Metabolism Elimination

Pronexus

Lead optimization in vivo

PK/PD
microdialysis
biosensors
analytical
neurochemistry

Animal models

"Go-No Go" decision

Rodent models

Psychiatric and neurodegenerative diseases

- Depression, ADHD
- Schizophrenia
- Drug abuse liability, NPS
- Parkinson´s disease
- Alzheimer´s disease, cognitive function
- Huntington´s disease

Metabolic and rare disorders

- Gaucher disease, Niemann-Pick type C disease
- Hepatic encephalopathy, CYP450-selective probe drugs

Pronexus Analytical - core expertise

Preclinical research: Experimental models

- Microdialysis protocols on anesthetized or awake rats and mice
- Microsurgery, stereotaxic surgery on small rodents
- Simultaneous microdialysis and behavioral monitoring
- Targeted pharmacokinetics and drug brain delivery
- Behavioral tests

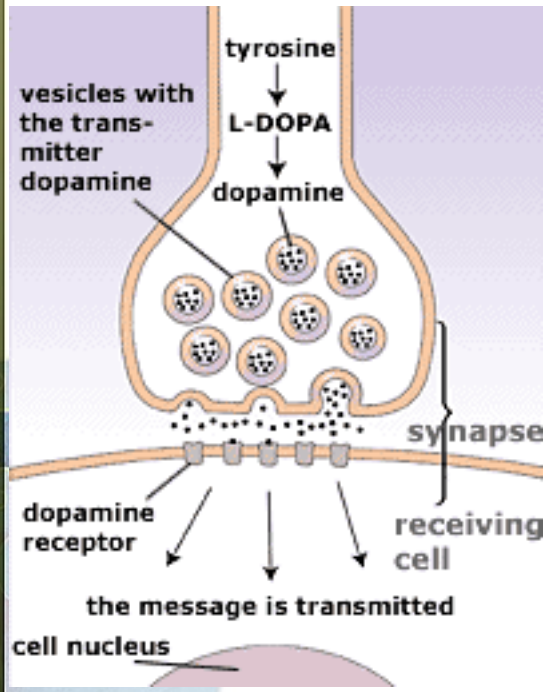
Analytical services

- UHPLC-MS/MS techniques optimized for determination of neurotransmitters, neuromodulators, metabolites and related molecules in the microdialysates and other biological samples
- UHPLC-MS/MS for PK/TK studies
- Chiral separations
- HPLC methods with electrochemical, fluorescence, UV, conductivity detection
- ELISA – VIS, FL, LM, TRF mode
- Enzymatic spectrophotometric assays for clinical microdialysates

Historical link between microdialysis and dopamine research

Nobel Prize in Physiology or Medicine 2000
for discovery of the neurotransmitter dopamine

Prof. Arvid Carlsson



Prof. Urban Ungerstedt

Prof. Urban Ungerstedt who pioneered the microdialysis technique and a member of the Nobel Committee at that time, delivering the Presentation Speech for the 2000 Nobel Prize in Physiology or Medicine at the Stockholm Concert Hall.

Analytical services

HPLC, UHPLC-MS/MS

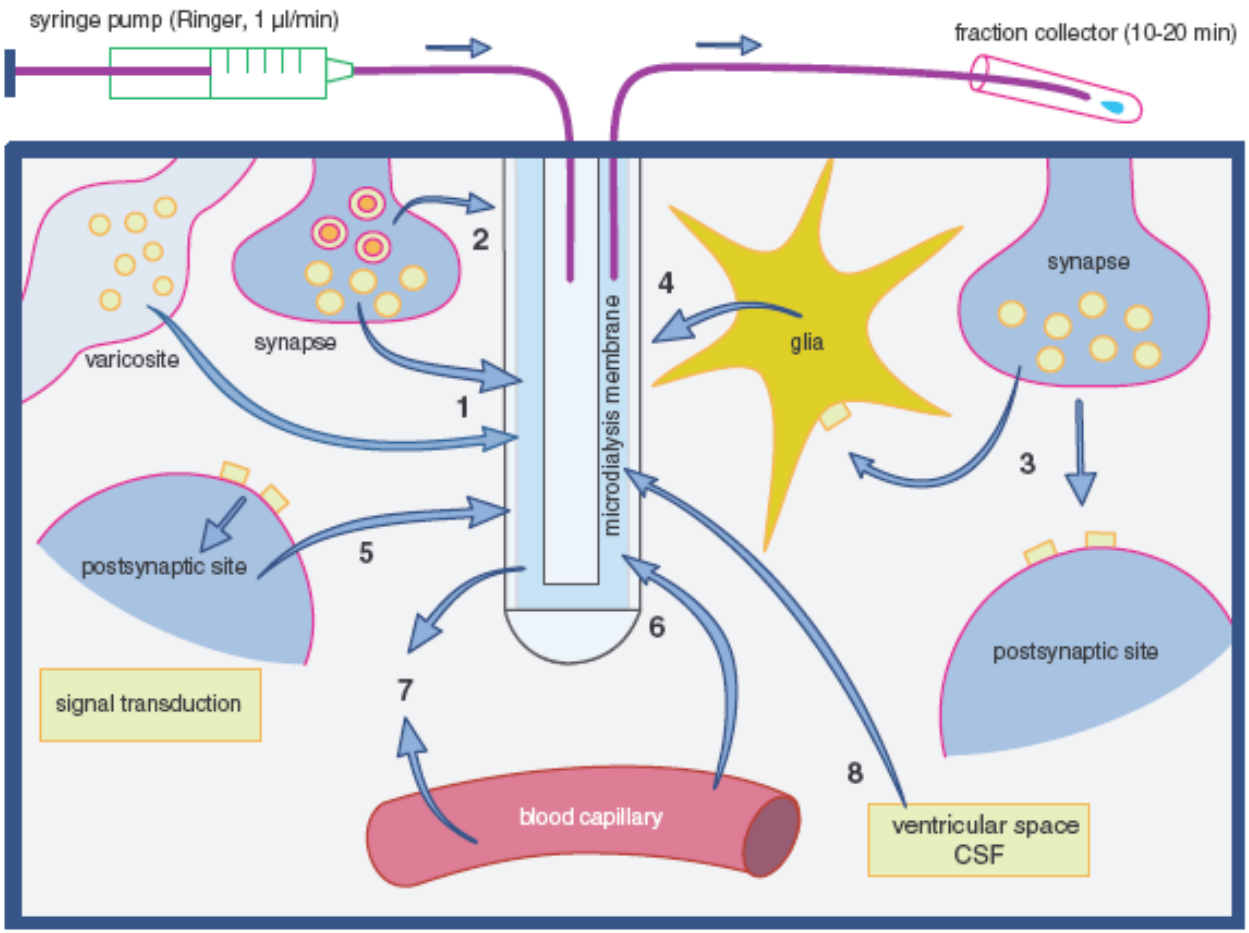
Small-molecular test compounds, pharmaceuticals, nutraceuticals, toxins
Monoamines: Dopamine, Noradrenaline, Serotonin, Histamine
Acetylcholine, Choline
Amino acids: Glu, Asp, GABA, Gly, D-Ser, physiological AAs
Adenosine (and other purines), cAMP
Kynurenine, Kynurenic acid, Quinolinic acid, related metabolites
Reactive oxygen species (ROS)
Glucose, lactate, pyruvate, glycerol
Amino acids - primary and secondary
Gaseous messengers: NO (NO_2^-, NO_3^-)

Hexosylsphingolipids: GlcCer, GalCer, GlcSph, GalSph

EIA, ELISA

Neuropeptides; proteins - fragments; soluble forms of Aβ peptides
Cytokines, interleukins, trophic factors, hormones, growth factors

Sampling molecules in the brain microenvironment by microdialysis



1,2,3. Presynaptic levels - release of neurotransmitters, neuromodulators and neuropeptides.

3, 4. Neuron-glia interactions - sampling glutamate, GABA, interleukins, trophic factors.

5. Signal transduction - monitoring second messengers such as cAMP, cGMP.

6. Molecules transported from blood capillaries - glucose, nutrients, drugs.

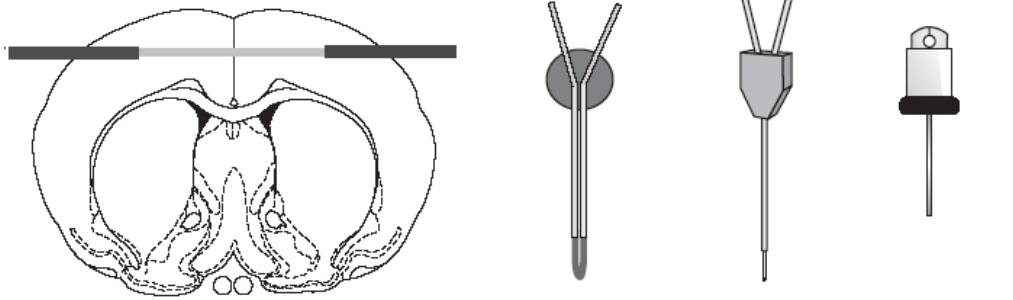
7. Neuro-vascular communication, NO, nitrite, nitrate.

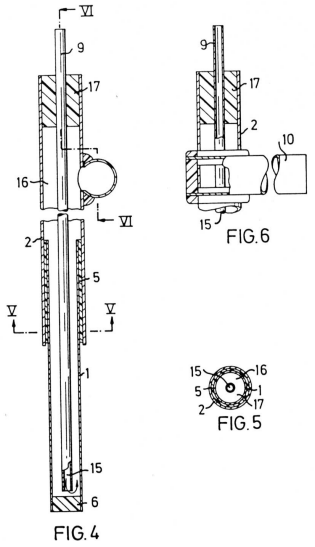
8. Molecules transported from, or into the CSF.

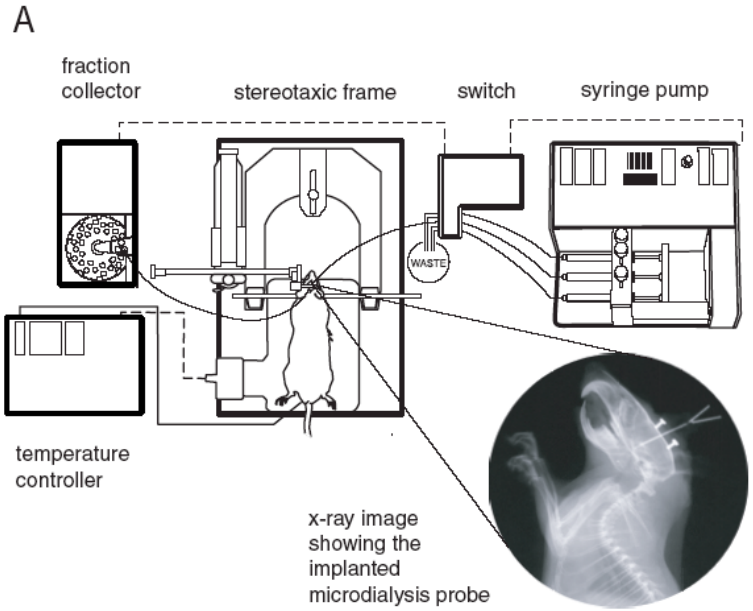
J. Kehr and T. Yoshitake, In: Encyclopedia of sensors, Ed.G.A. Grimes, ACS (2006) Vol 6, pp. 287-311.

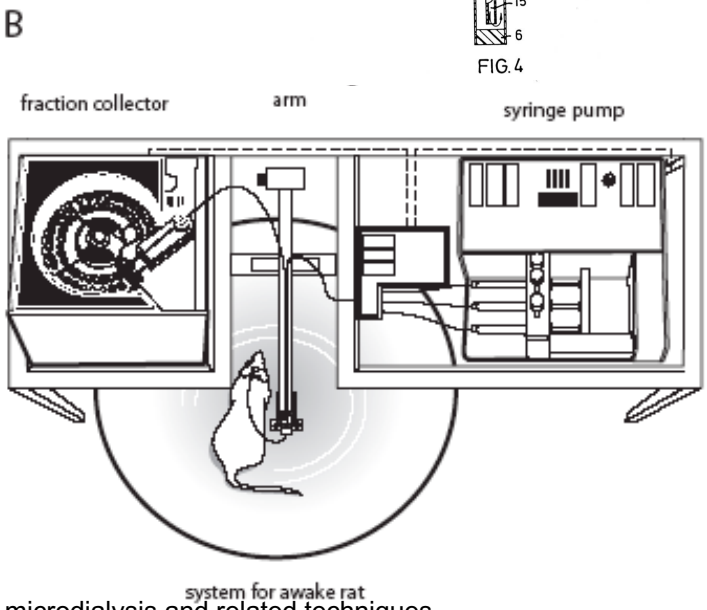
Instruments and devices for microdialysis

CMA/10







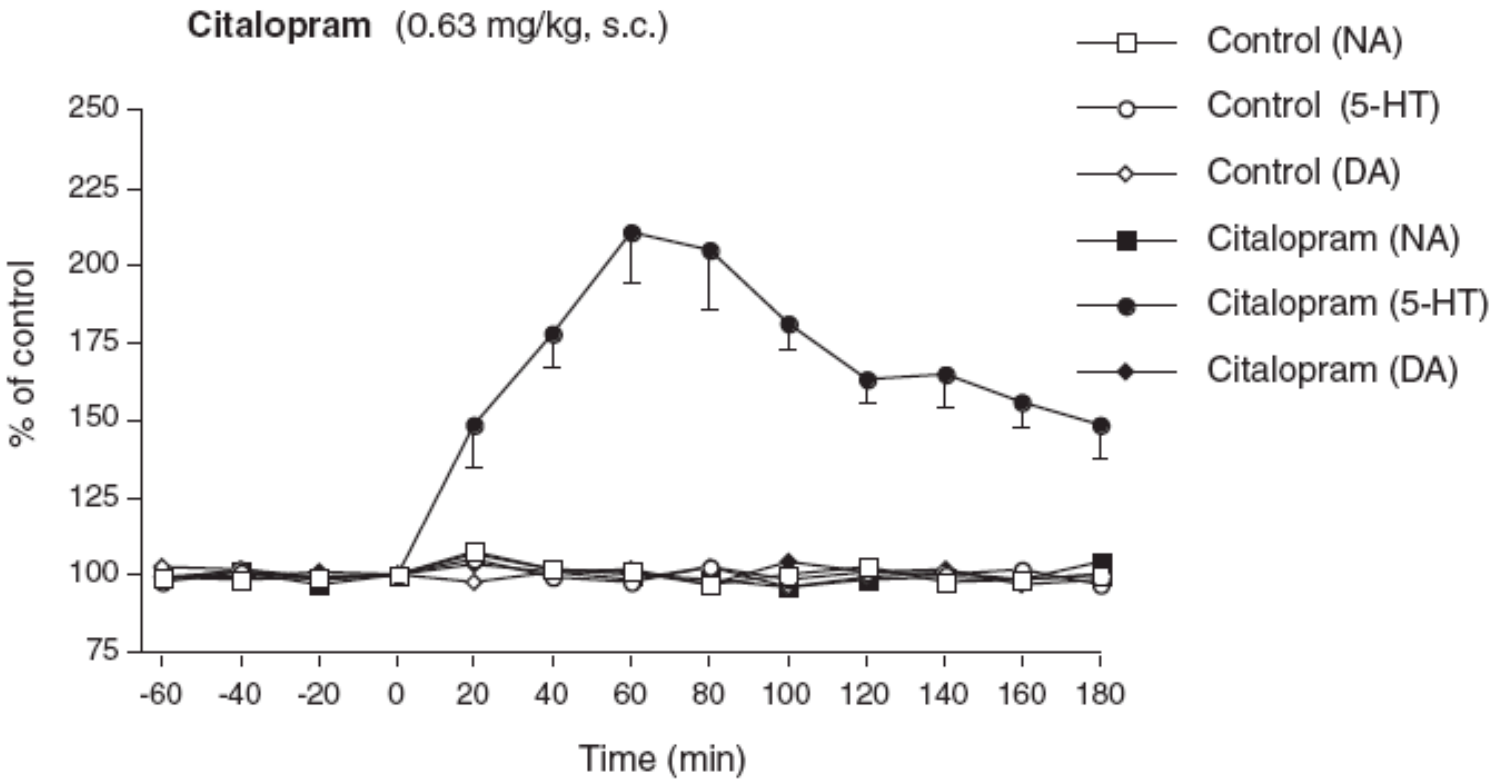


Kehr J. (1999) Monitoring chemistry of brain microenvironment: biosensors, microdialysis and related techniques. Chapter 41. In: Modern techniques in neuroscience research. (Eds. U. Windhorst and H. Johansson) Springer-Verlag GmbH., Heidelberg, Germany. 1149-1198.

Microdialysis - a major tool in functional pharmacology

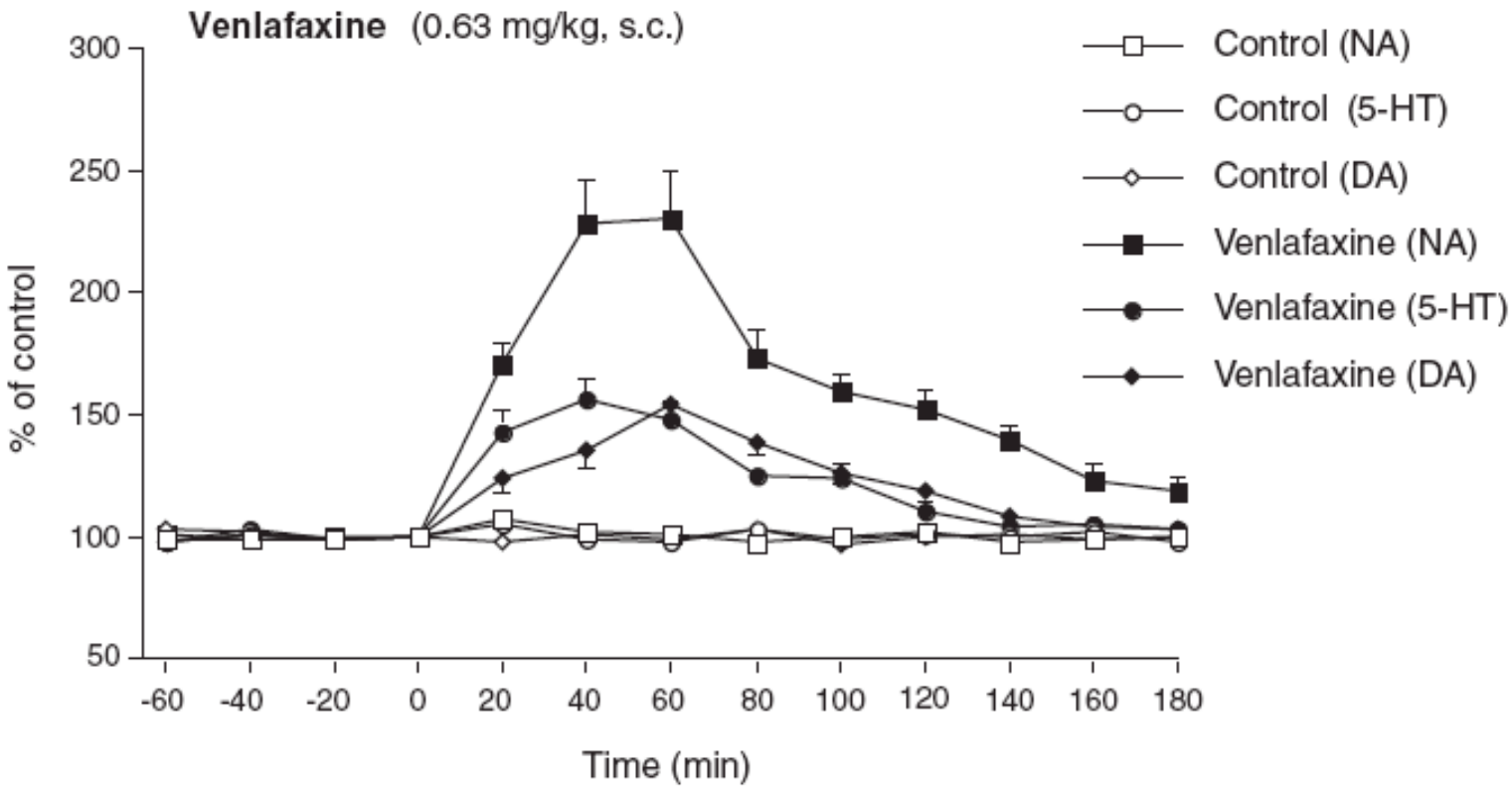
- accelerates the process of drug discovery and strengthens functional validation of candidate drugs

Effect of chronic (14 days/once daily) treatment with SSRI citalopram on levels of monoamines NA, DA and 5-HT in the mPFC of awake rat



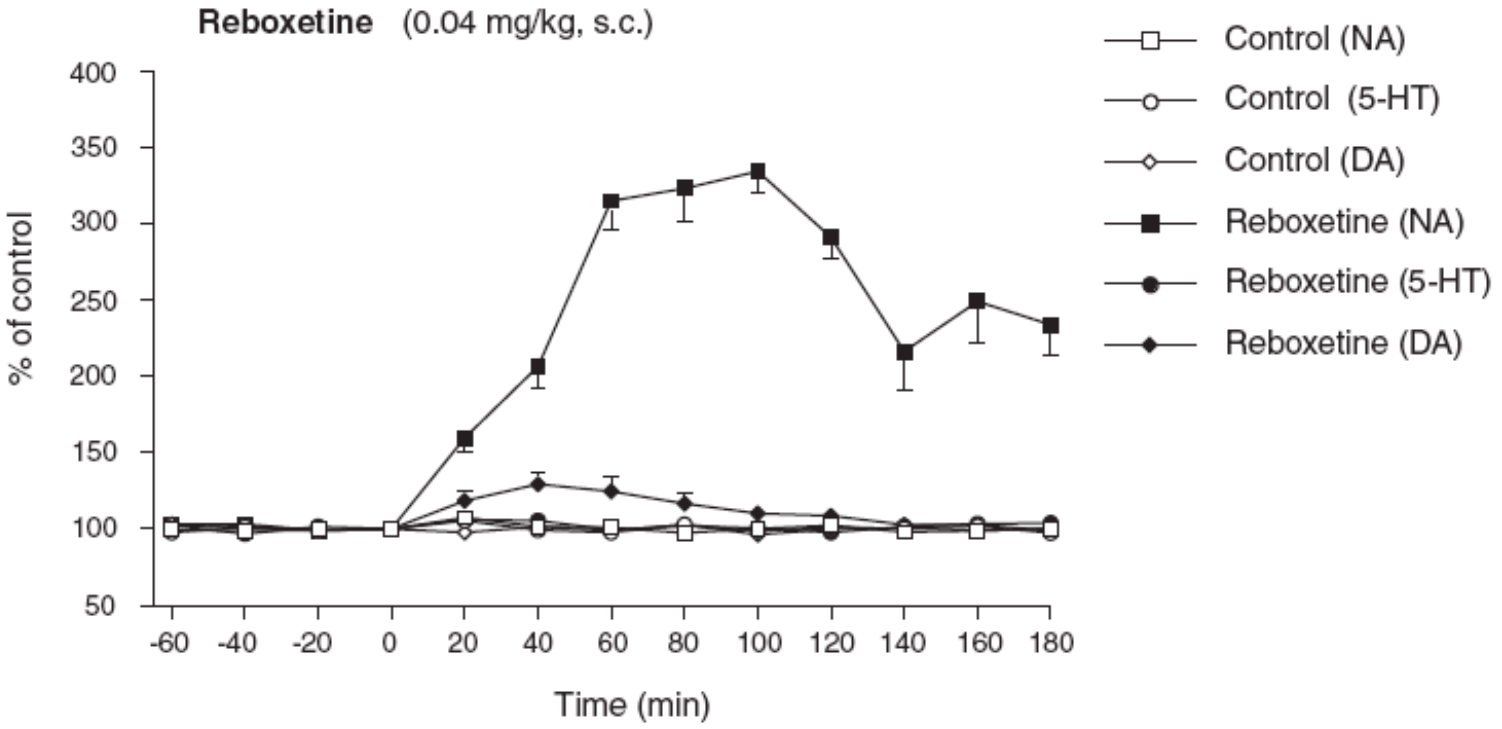
Microdialysis
- a major tool in functional pharmacology

Effect of chronic (14 days/once daily) treatment with NSRI venlafaxine on levels of monoamines NA, DA and 5-HT in the mPFC of awake rat

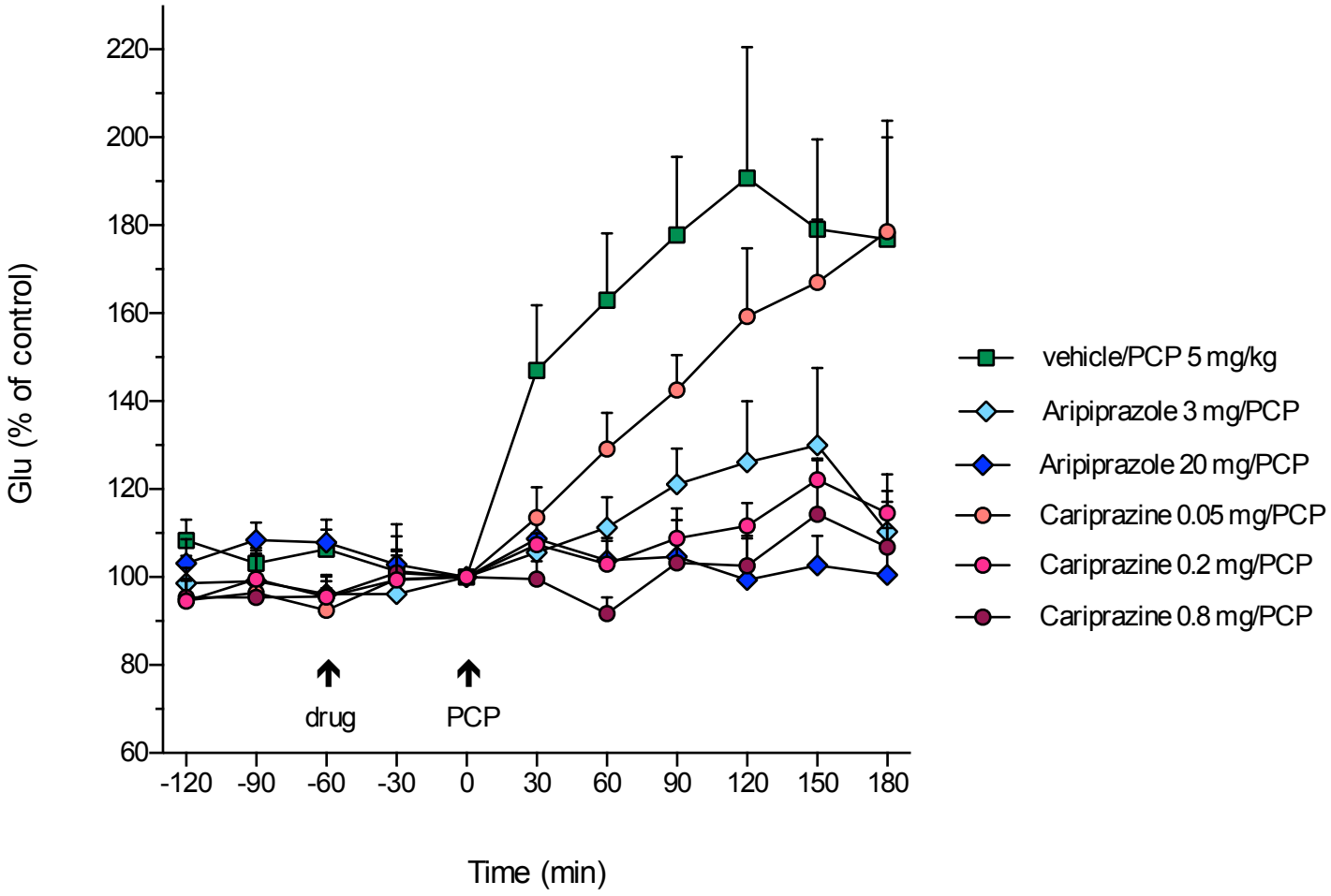


Microdialysis
- a major tool in functional pharmacology

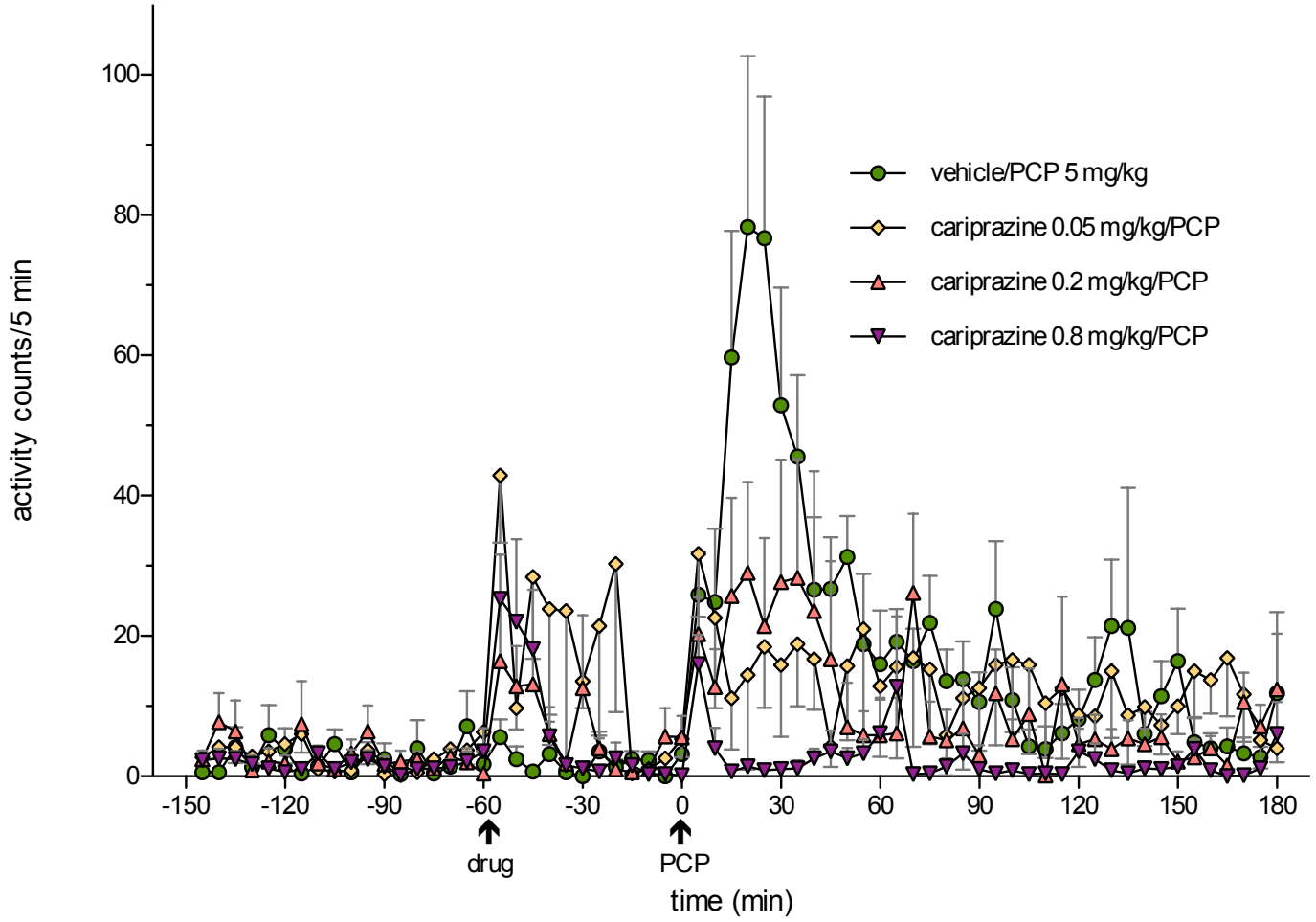
Effect of chronic (14 days/once daily) treatment with NRI reboxetine on levels of monoamines NA, DA and 5-HT in the mPFC of awake rat



Phencyclidine rat model of schizophrenia: Effects of aripiprazole and cariprazine on extracellular levels of glutamate in mPFC of awake rat

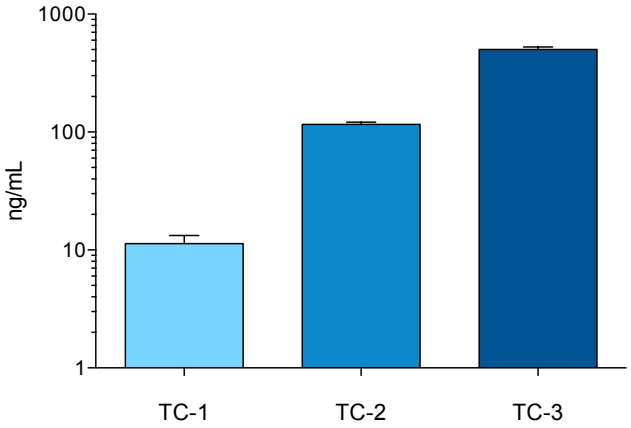


Effect of cariprazine (Vraylar) on PCP-induced increase in locomotor activity

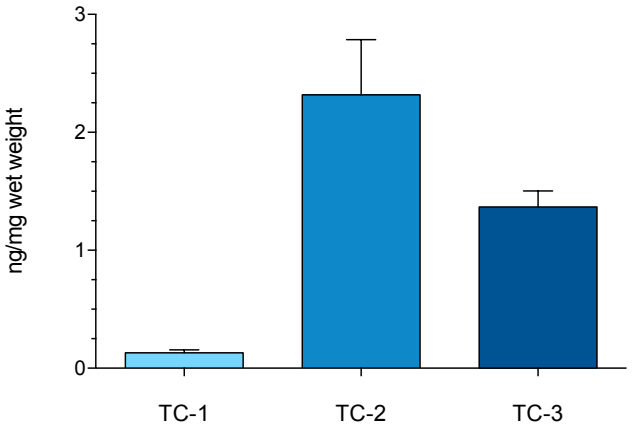


PK/PD study of three test compounds by use of dual-probe microdialysis

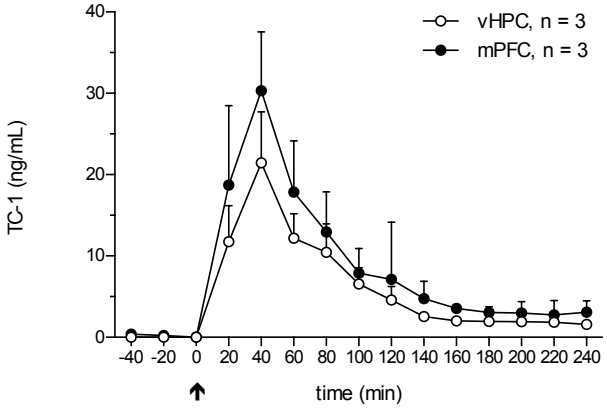
Plasma concentrations of 3 test compounds (TC)
4 hours after 30 mg/kg p.o. administration in each group



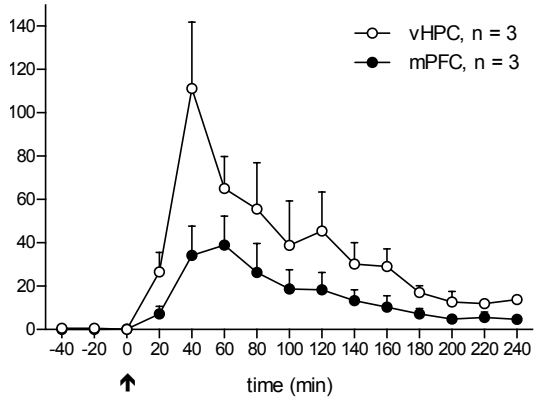
Concentrations of 3 test compounds (TC) in rat frontal cortex
4 hours after 30 mg/kg p.o. administration in each group



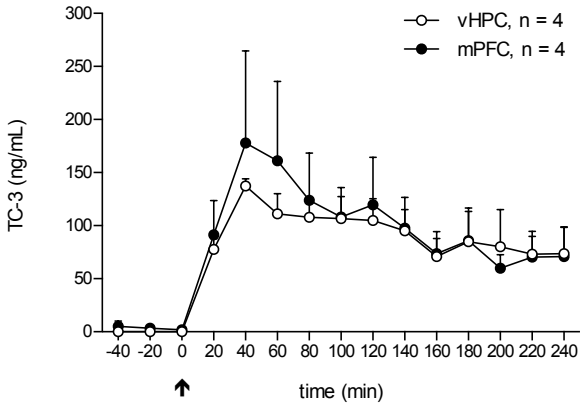
Concentrations of TC-1 in the microdialysates from rat vHPC and mPFC



centrations of TC-2 in the microdialysates from rat vHPC an

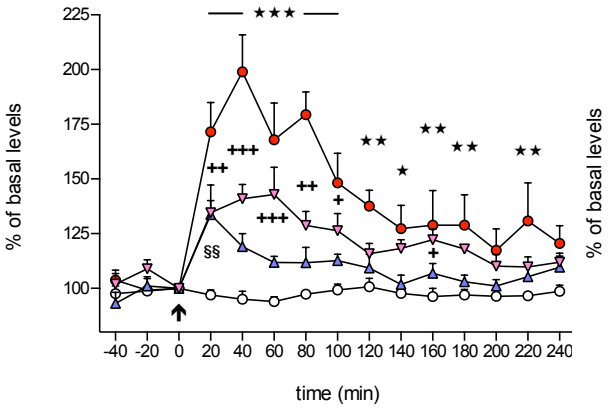


Concentrations of TC-3 in the microdialysates from rat vHPC and mPFC

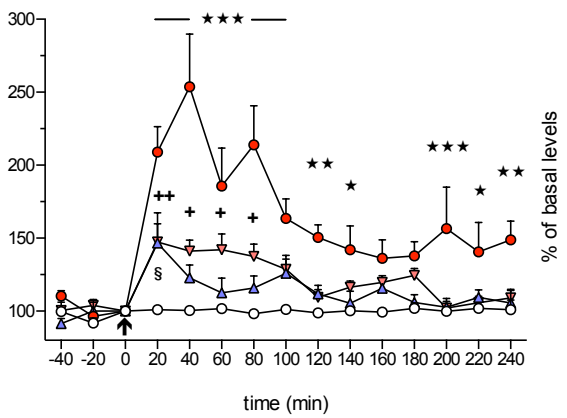


PK/PD study of three test compounds by use of dual-probe microdialysis

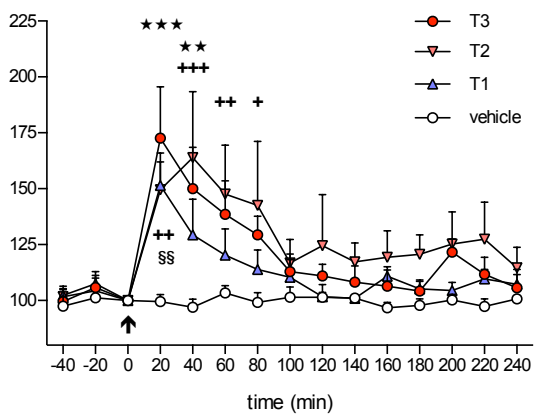
DA levels in the rat mPFC



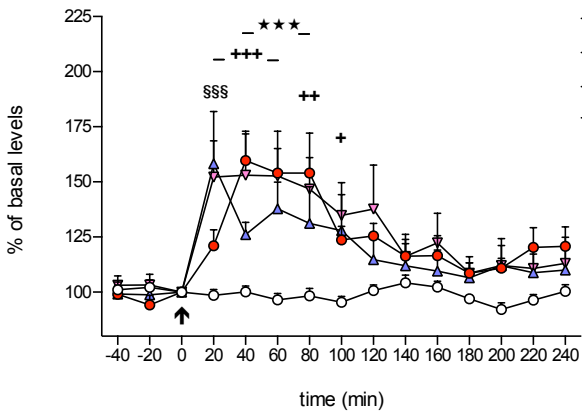
NA levels in rat mPFC



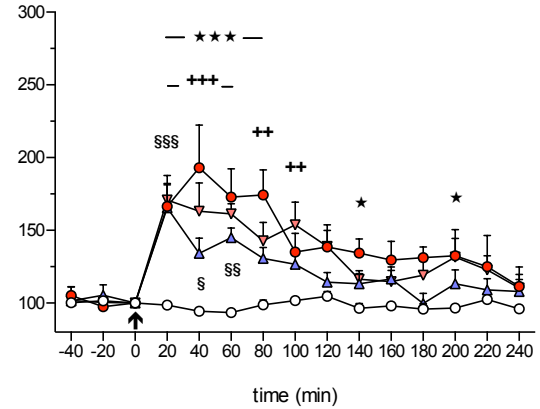
5-HT levels in rat mPFC



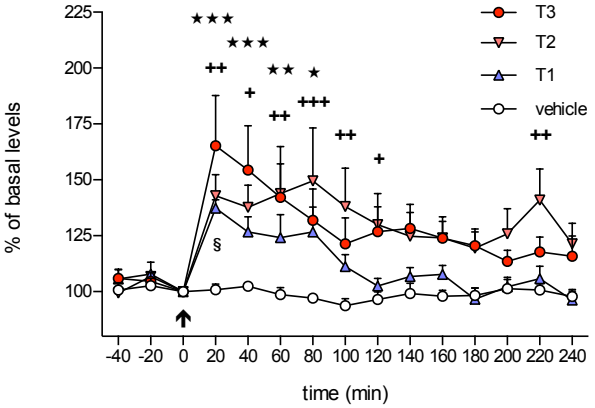
DA levels in the rat vHPC



NA levels in rat vHPC



5-HT levels in rat vHPC



Poster presented at the ACNP meeting, Florida, Dec 4-8, 2016

W64

Rapastinel (Glyx-13), a Rapid Acting Antidepressant, Does Not Increase Extracellular Levels of Dopamine and Glutamate in Rat Medial Prefrontal Cortex

Pradeep Banerjee[1], John Donello[1], Takashi Yoshitake[2], Jan Kehr[3]

[1]Allergan, Inc., Jersey City, New Jersey, USA; [2]Karolinska Institutet, Department of Physiology and Pharmacology, Stockholm, Sweden; [3]Pronexus Analytical AB, Stockholm, Sweden

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ABSTRACT

Background: Rapastinel (Glyx-13) is a novel NMDA receptor modulator with glycine-like partial agonist properties. Rapastinel is in late-stage clinical development as an adjunct therapy for major depressive disorder. Ketamine, a noncompetitive NMDA receptor channel blocker, is known to produce rapid and sustained antidepressant effect in treatment-resistant patients with major depression. However, ketamine, a schedule II controlled substance, is abused frequently and is known to produce addictive and psychotomimetic effects in animals and humans. Both ketamine and rapastinel produce antidepressant-like effects in rodent models of depression but rapastinel does not exhibit ketamine-like CNS adverse effects. For example, rapastinel does not produce ketamine-like sedation and rewarding effects or disrupt sensorimotor gating in rodents (Burgdorf et al, 2013). In the present study, we evaluated acute effects of rapastinel and ketamine on extracellular levels of dopamine and glutamate in rat medial prefrontal cortex (mPFC) using intracerebral microdialysis. The onset and the duration of antidepressant-like effects of rapastinel and ketamine were evaluated in the rat forced swim test (FST).

Methods: All experiments were carried out in adult male Sprague Dawley rats. Microdialysis experiments were carried out in freely moving rats as described in Kehr et al 2001. Briefly, guide cannulae were surgically implanted into mPFC using standard stereotaxic coordinates. On the day of dialysis, a 3-mm semipermeable probe (molecular weight cut off: 50 kDa) was inserted into the guide cannula and mPFC was dialyzed with artificial CSF (flow rate: 1 uL/min) for at least 2 hr. Following this, baseline dialysate samples were collected for the next 60 min and then for additional 3 hr following drug administration. Dopamine and glutamate concentrations in the dialysates were measured using HPLC and LC-MS/MS methods. Rat FST was carried out using a modified version of Porsolt (Porsolt, 1979) at 1-hr, 2-day and 7-day postdose.

Results: Both rapastinel (3-30 mg/kg) and ketamine (3-30 mg/kg) decreased immobility time in the rat FST when tested at 1-hr postdose. This antidepressant-like effect of both drugs persisted at day 2 and at day 7. In the dialysis assay, ketamine significantly elevated extracellular levels of dopamine and glutamate in the mPFC within the first 60 min of dosing. By contrast, rapastinel did not increase dopamine or glutamate levels in mPFC at any of the doses tested.

Conclusion: These data provide a potential neurochemical basis for rapastinel's favorable CNS adverse effects compared to ketamine. Increase in brain dopamine and glutamate levels by ketamine may play a significant role in its high abuse potential and psychotomimetic-like effects.

Figure 1: Both rapastinel and S-ketamine dose-dependently reduced sustained antidepressant-like effects in the rat FST model after a single dose; the antidepressant effects of rapastinel and S-ketamine were measured at 60 min postdose (solid bars) and 7 days postdose (shaded bars).

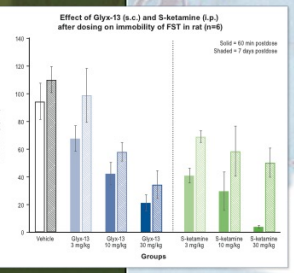


Figure 2: Kinetics of rapastinel in the extracellular space (ECS) of rat medial prefrontal cortex (mPFC) after a single SC dose of rapastinel (3 or 10 or 30 mg/kg). ECS levels of rapastinel increased within 20 min postdose and dissipated rapidly (within 60-80 min postdose). Note: the antidepressant effects of rapastinel was measured at 60 min and 7 days postdose. The ECS-C_{max} of rapastinel at 10 or 30 mg/kg is ~10 ng/mL (25 nM) or ~40 ng/mL (98 nM); in the calcium imaging studies, NMDAR activation by rapastinel is observed at 30-300 nM, suggesting that the antidepressant effect of rapastinel is mediated by NMDAR activation. Rapastinel in the dialysate was measured using a HPLC-MS/MS method.

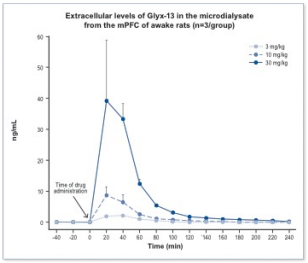


Figure 3: Representative photomicrograph of a dialysis probe placed in mPFC of a rat (panel A); panel B shows a schematic illustration of a dialysis probe in the mPFC area (cingulate cortex = Cg1; perilimbic cortex = PrL; infralimbic cortex = IL)

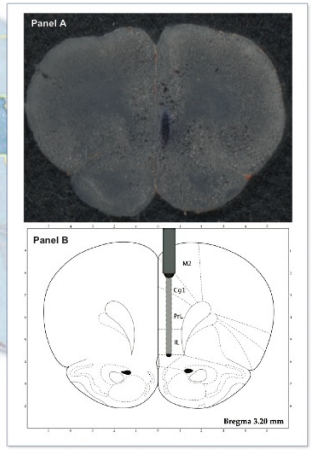


Figure 4A: S-ketamine (30 mg/kg) significantly increased dopamine (DA) levels in rat mPFC (evaluated as % increase over baseline in figure 4A and relative $AUC_{0\text{-}240min}$ in figure 4B using intracerebral microdialysis in freely moving rats). Rapastinel (3, 10 or 30 mg/kg) did not increase DA levels in mPFC. These data suggest that rapastinel does not possess ketamine-like rewarding effects and may provide a mechanistic basis for our earlier observation that rapastinel lacks reinforcing potential in monkeys trained to abuse cocaine (unpublished data); ###p< 0.01

Figure 4B: Extracellular levels of DA in mPFC (Relative $AUC_{0\text{-}240min}$) (%); ###p< 0.01

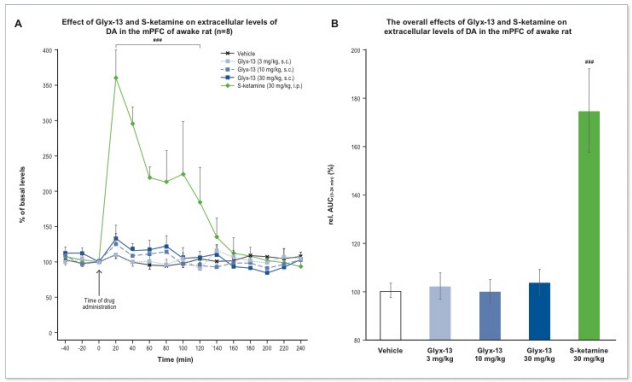


Figure 5A: S-ketamine (30 mg/kg) produced significant increases in 5-HT levels in mPFC (evaluated as % increase over baseline in figure 5A and relative $AUC_{0\text{-}240min}$ (%) in figure 5B using intracerebral microdialysis in freely moving rats). Rapastinel (3, 10 or 30 mg/kg) did not increase 5-HT levels in mPFC; ###p< 0.01

Figure 5B: Extracellular levels of 5-HT in mPFC (Relative $AUC_{0\text{-}240min}$) (%); ##p< 0.05

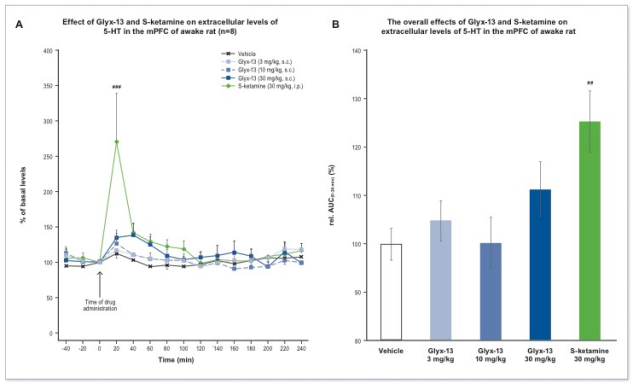


Figure 6A: S-ketamine (30 mg/kg) tended to increase glutamate (GLU) levels in mPFC (evaluated as % increase over baseline in figure 6A and relative $AUC_{0\text{-}240min}$ (%) in figure 6B using intracerebral microdialysis in freely moving rats). Rapastinel (3, 10 or 30 mg/kg) did not increase GLU levels in mPFC. These data provide a mechanistic explanation for the observed psychotomimetic-like effects of ketamine, and lack of such effects by rapastinel in rodents (Moskal et al., 2014)

Figure 6B: Extracellular levels of GLU in mPFC (relative $AUC_{0\text{-}240min}$ (%)

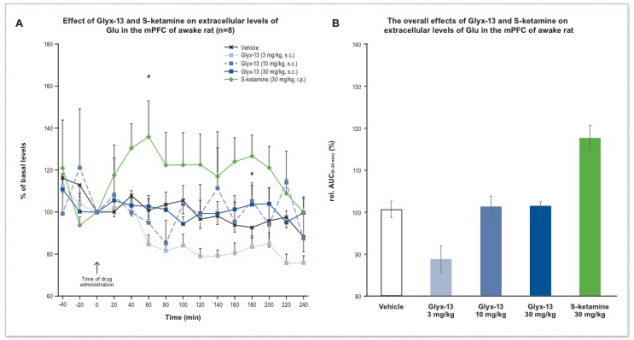


Figure 7: Neither S-ketamine (30 mg/kg) nor rapastinel (3, 10 or 30 mg/kg SC) altered the extracellular levels of GABA in mPFC.

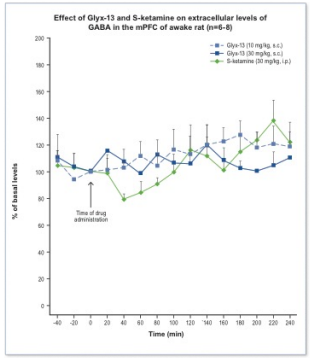
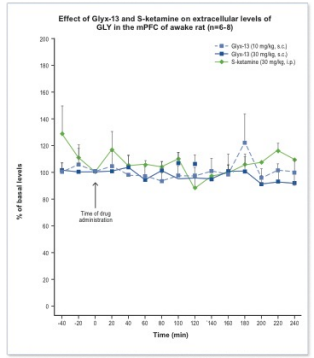


Figure 8: Neither S-ketamine (30 mg/kg) nor rapastinel (3, 10 or 30 mg/kg SC) altered the extracellular levels of glycine in mPFC.



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Moskal JR, Kuo AG, Weiss C, Wood PL, O'Connor Hanson A, Kotsi S, Harris RB, Disterhoft JF (2005) GLYX-13: a monoclonal antibody-derived peptide that acts as an N-methyl-D-aspartate receptor modulator. Neuropharmacology, 48(7):1077-1087.

Moskal JR, Burch R, Burgdorf JS, Kroes RA, Stanton PK, Disterhoft JF, Leander JD (2014). GLYX-13, an NMDA receptor glycine site functional partial agonist enhances cognition and produces antidepressant effects without the psychotomimetic side effects of NMDA receptor antagonists. Expert Opin Investig Drugs, 23(2):243-254.

Zhang XL, Sullivan JA, Moskal JR, Stanton PK (2008) A NMDA receptor glycine site partial agonist, GLYX-13, simultaneously enhances LTP and reduces LTD at Schaffer collateral-CA1 synapses in hippocampus. Neuropharmacology, 55(7):1238-1250.

DISCLOSURE

This study was funded by Allergan (Irvine, CA). Graphics support was provided by Prescott Medical Communications Group (Chicago, IL), a contractor of Allergan. All authors met ICMJE authorship criteria. Neither honoraria nor payments were made for authorship. Financial arrangements of the authors with companies whose products may be related to the present report are listed below, as declared by the authors. P. Banerjee and J. Donello are full-time employees of Allergan. T. Yoshitake is a full-time employee Karolinska Institutet. J. Kehr is a full-time employee of Pronexus Analytical AB.

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- www.allergancongressposters.com/805512.

Poster presented at the ACNP meeting, Phoenix, Arizona, December 8-11, 2024

Didesmethyl-Cariprazine, the Major Active Human Metabolite of Cariprazine With Greater Dopamine D_3 vs D_2 Receptor Selectivity, Increases Dopamine Release in the Rat Medial Prefrontal Cortex

Jan Kehr,[1] Bence Farkas,[2] Viktor Román,[2] Attila Horváth,[2] Balázs Lendvai,[2] Nika Adham,[3]*

[1]*Pronexus Analytical AB, Bromma, Sweden;* [2]*Gedeon Richter Plc, Budapest, Hungary;* [3]*AbbVie Inc, Florham Park, NJ, USA*

** Presenting Author*

OBJECTIVE

To evaluate whether there are any differences in the neurochemical effects of cariprazine (CAR) in comparison with didesmethyl-CAR (DDCAR) to provide potential mechanism(s) that can account for the unique clinical profile of CAR

CONCLUSIONS

DDCAR produced a significant increase in the level of dopamine (DA) and DA metabolites in the medial prefrontal cortex (mPFC), whereas CAR did not show any significant effects on these parameters with a trend towards a decrease at the lower dose tested

These data suggest that DDCAR's greater D_3 receptor affinity and selectivity over D_2 receptor compared to CAR may contribute to the increase in DA neurotransmission in the mPFC, supporting the notion that the increasing D_3 vs D_2 selectivity of compounds produces more benefit for ameliorating negative and cognitive symptoms

DDCAR treatment did not have any impact on acetylcholine (ACh) and histamine (HA) levels, whereas CAR resulted in a significant decrease in these neurotransmitters, which may be due to the CAR's greater D_2 receptor activity compared to DDCAR

Collectively, these results demonstrate a differential neurochemical profile of DDCAR in comparison to CAR, indicating that the predominance of this metabolite following stable dosing of CAR may contribute to the unique clinical profile of CAR therapy in various psychiatric symptoms

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INTRODUCTION

Cariprazine

- Cariprazine (CAR) is a dopamine (DA) D_3-preferring D_3/D_2 and serotonin 5-HT_{1A} receptor partial agonist approved by the US Food and Drug Administration to treat adults with schizophrenia, manic, mixed, and depressive episodes associated with bipolar I disorder as well as adjunctive treatment for major depressive disorder (MDD)
- CAR is metabolized to desmethyl-CAR (DCAR) and ultimately to didesmethyl-CAR (DDCAR) with DDCAR being the prominent moiety (~ 70% of total) at steady state in humans[1]
- Although in general, DDCAR displays comparable in vitro binding receptor profile and has a similar antipsychotic-like activity as CAR, this metabolite has greater D3 receptor (D_R) affinity and selectivity compared to the parent[2]

Study Objective

- To evaluate whether there are any differences in the neurochemical effects of CAR in comparison with DDCAR, which is more D_3 receptor-preferring, to provide potential mechanism(s) that can account for the unique clinical profile of CAR

Rationale for Analysis

- Neurochemical effects of CAR and DDCAR were evaluated in rats using microdialysis probing in the mPFC, a brain area of importance with dysregulated function in various psychiatric and cognitive disorders. The negative and/or cognitive symptoms of schizophrenia may be due to reduced dopaminergic and/or cholinergic tone in cortical brain areas[3,4]
- Alteration in the function of dopamine D_3 receptors may play a role in this cortical hypofunctionality and underlie the deficits in social behaviors and cognitive functions in various mood disorders[5,6]

METHODS

Analysis

- Neurochemical effects of CAR and DDCAR were evaluated in rats (male Sprague-Dawley rats weighing 300–320 g, N = 49) using microdialysis probing in the mPFC
- Seven days before the microdialysis experiment, the rats were pretreated with buprenorphine and carprofen, anesthetized with isoflurane, and the guide cannulas were stereotaxically implanted into the mPFC. On the day of experiment, the separate groups of awake rats were treated with CAR and DDCAR, and the samples were collected in 20-minute intervals for the following 4 hours
- Microdialysis samples concentrations of neurotransmitters DA, norepinephrine (NE), 5-HT, ACh, HA, glutamate (Glu), gamma-aminobutyric acid (GABA), glycine (Gly), as well as the levels of D-serine (D-Ser) and the monoamine metabolites, 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured by ultra high-performance liquid chromatography with electrospray tandem mass spectroscopy (UHPLC-MS/MS) following their derivatization with benzoyl chloride except for ACh which was measured by a separate UHPLC-MS/MS method. Concentrations of D-Ser were measured by UHPLC-MS/MS using a chiral separation column. Concentrations of CAR and DDCAR in the remaining volumes of the microdialysates were measured by UHPLC-MS/MS

Statistical Analysis

- The values were presented as mean ± standard error of mean (SEM) and differences were considered statistically significant at the $P < .05$ level
- The data was expressed as percentage of the basal concentrations and as relative area-under-the curve $AUC_{0\text{-}240\,min}$ values
- The mean basal levels in the control and treated groups and the relative $AUC_{0\text{-}240\,min}$ values were compared by use of one-way analysis of variance (ANOVA) followed by Tukey's and Dunnett's multiple comparison test, respectively
- Differences between the groups and treatments were analyzed by repeated measures two-way ANOVA followed by Bonferroni's posttest

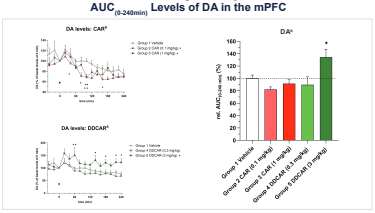
RESULTS

Basal Levels[a] of Neurotransmitters, Neuromodulators and Monoamine Metabolites in the mPFC of Awake Rats

	Group 1 Vehicle	Group 2 CAR 0.1 mg/kg	Group 3 CAR 1 mg/kg	Group 4 DDCAR 0.3 mg/kg	Group 5 DDCAR 3 mg/kg
DA	0.287 ± 0.0203	0.305 ± 0.0495	0.336 ± 0.0747	0.414 ± 0.0241	0.083 ± 0.0431*
NE	0.675 ± 0.0872	0.455 ± 0.0487	0.493 ± 0.1196	0.773 ± 0.0997	0.468 ± 0.1671
5-HT	1.304 ± 0.3596	0.472 ± 0.0635	0.521 ± 0.0749	0.91 ± 0.2649	0.438 ± 0.0275
ACh	3.682 ± 0.5321	3.483 ± 0.4804	4.505 ± 0.9361	3.722 ± 0.3429	3.245 ± 0.8793
HA	5.844 ± 0.3934	13.30 ± 4.681	6.131 ± 1.06	66.60 ± 22.72**	12.39 ± 2.694
Glu	353.5 ± 69.5	955.4 ± 244.5	1199 ± 418.1	523.6 ± 93.7	1196 ± 422.5
GABA	28.80 ± 6.91	42.56 ± 11.08	47.12 ± 5.01	54.39 ± 3.68	54.86 ± 11.56
Gly	3305 ± 1305	4098 ± 1416	7139 ± 1183	2408 ± 589	4611 ± 1093
D-Ser	2152 ± 168.9	2123 ± 160.4	1873 ± 155.5	1798 ± 304.7	2300 ± 95.5
MHPG	20.48 ± 2.821	27.26 ± 4.096	31.72 ± 4.073	23.09 ± 2.635	32.21 ± 4.585
DOPAC	43.17 ± 7.418	30.99 ± 3.948	58.29 ± 11.78	39.48 ± 7.938	52.19 ± 6.850
HVA	24.83 ± 4.162	26.85 ± 3.543	98.48 ± 37.18*	59.53 ± 10.99	111.5 ± 11.35*
5-HIAA	174.6 ± 35.45	212.8 ± 56.22	295.9 ± 81.69	171.2 ± 20.28	338.4 ± 45.71

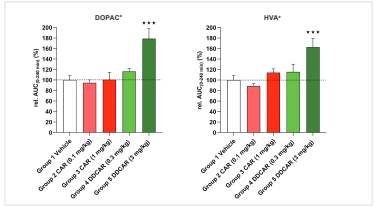
[a]The basal levels were calculated as a mean of 3 basal levels (-60 to 0 min) of each rat, thereafter as the mean ± SEM of these levels for each group and the analyte. The concentrations are expressed in nmol/L (nM). One-way ANOVA followed by Dunnett's multiple comparison test, (*) $P < .05$; (**) $P < .01$ as compared to the vehicle group.
5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; ACh, acetylcholine; CAR, cariprazine; DA, dopamine; DDCAR, didesmethyl-CAR; DOPAC, 3,4-dihydroxyphenylacetic acid; D-Ser, D-serine; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HA, histamine; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; mPFC, medial prefrontal cortex; NE, norepinephrine.

DDCAR but not CAR Significantly Increased the $AUC_{(0\text{-}240min)}$ Levels of DA in the mPFC



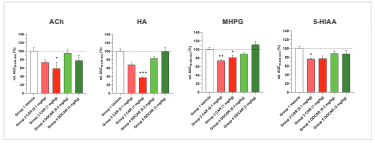
[a]Effects of CAR and DDCAR on extracellular levels of DA calculated as percentage of basal levels at 0 min (Mean ± SEM) in the mPFC of awake rats; two-way (mixed-effects model) ANOVA followed by Dunnett's multiple comparisons test; CAR: (●) $P < .05$; (●●) $P < .01$; (***) $P < .01$; DDCAR: (*) $P < .05$; (**) $P < .01$.
[b]Extracellular levels (Mean ± SEM) of DA in the mPFC of awake rats; one-way ANOVA followed by Dunnett's multiple comparisons test; (*) $P < .05$.
AUC, area under the curve; CAR, cariprazine; DA, dopamine; DDCAR, didesmethyl-CAR; mPFC, medial prefrontal cortex.

DDCAR but not CAR Significantly Increased the $AUC_{(0\text{-}240min)}$ Levels of DA Metabolites, DOPAC and HVA, in the mPFC



[a]Extracellular levels (Mean ± SEM) of DOPAC and HVA in the mPFC of awake rats; one-way ANOVA followed by Dunnett's multiple comparisons test; (***) $P < .001$.
AUC, area under the curve; CAR, cariprazine; DA, dopamine; DDCAR, didesmethyl-CAR; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; mPFC, medial prefrontal cortex.

CAR Caused Significant Decreases in the Neurotransmitters, ACh and HA, and Monoamine Metabolites, MHPG and 5-HIAA, Levels[a] in the mPFC



[a]Extracellular levels (Mean ± SEM) of ACh, HA, MHPG, and 5-HIAA in the mPFC of awake rats; one-way ANOVA followed by Dunnett's multiple comparisons test; (*) $P < .05$; (**) $P < .01$; (***) $P < .001$.
5-HIAA, 5-hydroxyindoleacetic acid; ACh, acetylcholine; AUC, area under the curve; CAR, cariprazine; DDCAR, didesmethyl-CAR; HA, histamine; MHPG, 3-methoxy-4-hydroxyphenylglycol; mPFC, medial prefrontal cortex.

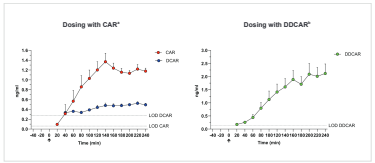
Summary[a] of CAR and DDCAR Effects in the mPFC

	CAR 0.1 mg/kg	CAR 1 mg/kg	DDCAR 0.3 mg/kg	DDCAR 3 mg/kg
DA	↓	-	-	↑
NE	↓	↓	-	-
5-HT	-	-	-	-
ACh	↓	↓	-	↓
HA	↓	↓	-	-
Glu	-	-	-	-
GABA	↓	-	-	-
Gly	-	-	-	-
D-Ser	-	-	-	-
MHPG	↓	↓	-	-
DOPAC	-	-	-	↑
HVA	-	-	-	↑
5-HIAA	↓	↓	-	-

[a]Effects of CAR and DDCAR on extracellular levels of neurotransmitters, monoamine metabolites and D-Ser in the mPFC of awake rats.The red (thick) arrows vs black (thin) arrows: the red arrows are statistically significant differences from the vehicle data for the relative $AUC_{0\text{-}240\,min}$ whereas the black arrows are trends. Statistical analysis: one-way ANOVA followed by Dunnett's multiple comparisons test; (*) $P < .05$; (**) $P < .01$.
5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; ACh, acetylcholine; AUC, area under the curve; CAR, cariprazine; DDCAR, didesmethyl-CAR; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; D-Ser, D-serine; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HA, histamine; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; mPFC, medial prefrontal cortex; NE, norepinephrine.

- Besides the observed effects on DA and its metabolites, the higher dose of CAR caused significant decreases in ACh, HA, MHPG, and 5-HIAA levels
- The levels of other analytes were only marginally decreased or not affected by any treatment

The Extracellular Levels of CAR and its Metabolite DCAR Gradually Increased in the mPFC and Remained Elevated Over the Entire 240-minute Sampling Period With DDCAR Being BLD



[a]Extracellular levels of CAR and DCAR in the mPFC of awake rats following treatment with CAR (1 mg/kg, peroral) at time 0 min. The levels of the second CAR metabolite, DDCAR, were not detectable when dosing with CAR.
[b]The levels of DDCAR were detectable in the mPFC following treatment with DDCAR (3 mg/kg, peroral) at time 0 min.
BLD, below the limit of detection; CAR, cariprazine; DCAR, desmethyl-cariprazine; DDCAR, didesmethyl-CAR; LOD, limit of detection; mPFC, medial prefrontal cortex.

Summary

Pronexus Analytical AB

- A unique portfolio of services including *in vivo* microdialysis and neurochemical analysis for evaluation of test substances in rodent models
- Expertise in pharmacology of major neurotransmitter systems, combined academic/industrial background
- International reputation, affiliation to Karolinska Institutet and Uppsala University
- A potential to expand, offering specialized PK/PD studies, *in vitro* and analytical CRO services
- Microdialysis in other organs/models: skin, muscle, liver, lung