

# **Challenges in Developing a Rat Model of Pharmacologically Induced Neurovascular Uncoupling**

**PhD thesis**

**Bence Tamás Varga**

Semmelweis University Doctoral College  
János Szentágothai Neurosciences Division



Supervisor:

István Gyertyán, Ph.D

Official reviewers:

Eszter Farkas, Ph.D

Péter Kovács, Ph.D

Head of the Complex Examination Committee:

György Bagdy, Ph.D

Members of the Complex Examination Committee:

Dóra Zelena, Ph.D

Viktor Román, Ph.D

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# 1. Introduction

## 1.1. The neurovascular unit

The neurovascular unit is a multicellular structure that facilitates tight coupling between vascular and neural activity in the brain. It regulates cerebral blood flow (CBF) in response to metabolic demands - termed neurovascular coupling (NVC). NVC ensures that localized increases in neuronal activity trigger rapid adjustments in blood supply, matching oxygen and nutrient delivery to metabolic needs. This process involves coordinated interactions among neurons, astrocytes, pericytes, microglia, and vascular endothelial cells, which collectively detect synaptic activity and translate it into vasodilation or constriction.

Endothelial cells form brain capillaries via tight junctions, creating the blood-brain barrier. They regulate molecular exchange and vascular tone through factors like nitric oxide (NO), epoxyeicosatrienoic acids (EETs), and prostaglandins. Pericytes control capillary diameter and CBF while stabilizing the barrier via platelet-derived growth factor receptor- $\beta$  signalling. Astrocytic endfeet release NO, prostaglandins,  $K^+$ , and arachidonic acid metabolites to mediate NVC. Neurons release NO and induce vasodilation, enhancing blood flow to active brain regions, while microglia survey for injury, releasing cytokines and ROS upon activation. The basal membrane and extracellular matrix provide structural support and regulate signalling via integrins.

Neurovascular uncoupling (NVU) occurs when this mechanism fails, leading to mismatches between metabolic demand and blood supply, impairing brain function. It is associated with stroke, epilepsy, Alzheimer's disease, traumatic brain injury, and old age.

## **1.2. Diseases linked to neurovascular unit dysfunction**

Dysfunction of the neurovascular unit is associated with several neurological disorders. **Alzheimer's disease** is characterized by early cerebrovascular dysfunction, with reduced CBF in memory-critical regions. Amyloid-beta peptides induce pericyte contraction, while reactive oxygen species damage endothelial cells, reducing NO bioavailability. Over time, this persistent mismatch between neuronal energy demand and blood supply accelerates synaptic failure and neurodegeneration.

**Aging** itself leads to progressive neurovascular deterioration. Endothelial cells exhibit reduced NO production, pericytes are gradually lost, and astrocytes shift toward a pro-inflammatory state, contributing to cognitive decline.

**Chronic hypertension** and **diabetes mellitus** promote cerebral small vessel disease through arteriolosclerosis, pericyte loss, and oxidative stress, leading to reduced vascular compliance, blunted functional hyperaemia, and increased risk of vascular cognitive impairment.

## **1.3. Lack of NVU targeting approach in antidementia drug research**

The pace of developing novel cognitive enhancers has been slow. Current Alzheimer's therapeutics are dominated by symptomatic treatments with limited benefits. While recent monoclonal antibodies targeting amyloid-beta represent a shift toward disease-modifying approaches, their clinical benefits are modest and effects on neurovascular function are not fully elucidated.

Despite identification of numerous molecular pathways in NVU, clinical translation has been limited. Endothelial NOS-targeted therapies have not advanced considerably, and pericyte-stabilizing strategies have not

reached clinical trials. A fundamental cause may be the lack of predictive preclinical models of NVU usable for drug evaluation.

#### **1.4. The need for preclinical NVU models**

Preclinical dementia research has been dominated by genetic models of amyloidosis or tauopathy, which are not optimized for studying NVU. Chronic hypoperfusion models induce vascular insufficiency but lack temporal control. The field lacks a standardized, pharmacologically tractable model for inducing NVU to evaluate drugs targeting cerebral haemodynamics.

Tarantini et al. in 2017 established proof-of-concept in mice, inducing NVU through a 7-day regimen inhibiting three key vasodilatory pathways:

- EETs (inhibited by MS-PPOH, 20 mg/kg/day)
- NO (inhibited by L-NAME, 100 mg/kg/day)
- Prostaglandins (inhibited by indomethacin, 7.5 mg/kg/day)

This treatment resulted in approximately 2/3 decrease in evoked functional hyperaemia and significant cognitive impairments in certain tasks. However, no validated procedure had been established in rats.

#### **1.5. The rodent test battery system**

Our research team developed an advanced cognitive test battery for rats evaluating multiple cognitive domains: attention (5CSRTT), social cognition (cooperation), spatial memory (MWM), procedural memory (pot jumping), and visual memory (pairwise discrimination). Animals are kept under controlled food restriction to maintain motivation. This system enables longitudinal assessment of cognitive impairment and potential treatments within the same cohort. Since NVU impairs

cognition in patients, we sought to implement it as an impairment method with strong neurobiological rationale.

## **2. Objectives**

The primary aim was to establish a pharmacologically induced NVU model in rats using MS-PPOH, L-NAME, and indomethacin, and validate this protocol in aged, experienced rats within our cognitive test battery.

Specific objectives:

1. Establish NVU-inducing pharmacological doses that effectively disrupt neurovascular coupling while minimizing side effects
2. Identify cognitive assays sensitive to NVU-induced impairment
3. Validate the NVU model in aged, cognitively experienced rats
4. Optimize treatment protocols through iterative experimentation

## **3. Methods**

### **3.1. Housing**

Animals were housed under reversed light-dark cycle, temperature-controlled room (20–23 °C), with water ad libitum and environmental enrichment. The necessary ethical permits were obtained.

### **3.2. Experiment 1: Dose-finding in young, naïve Wistar rats**

**Subjects:** 28 male Hannover Wistar rats (200-225 g)

**Treatment:** 5 mg/kg MS-PPOH, 10 mg/kg L-NAME, 1 mg/kg indomethacin, injected i.p. twice daily for 8 consecutive days, dissolved in 45 % HP $\beta$ CD. Control animals received vehicle.

#### **Cognitive assays:**

- Fear conditioning (Day 4 acquisition, Day 8 retention)
- Morris water maze (Day 5-7, three trials/day)

**CBF measurement:** In a terminal surgery, Laser Doppler probe measured changes in barrel cortex while contralateral whisker pad was stimulated. Coupling parameter calculated as mean stimulation amplitude minus baseline.

**Blood Pressure:** Measured using CODA® tail-cuff apparatus on Days 2, 5, and 7.

**Tissue Collection:** Barrel cortex and small intestine collected for prostaglandin E2 (PGE<sub>2</sub>) ELISA.

### **3.3. Experiment 2: Further dose refinement**

**Subjects:** 17 male Hannover Wistar rats (155-215 g, 2 months old)

**Treatment:** 2.5 mg/kg MS-PPOH, 5 mg/kg L-NAME, 0.5 mg/kg indomethacin, injected i.p. twice daily for 9 days, dissolved in 30 % HP $\beta$ CD.

#### **Cognitive assays:**

- Y-maze alternation (baseline, Day 4, Day 8)
- Lever-press learning (Days 6-8)
- Novel object recognition (Day 9)

**Measurements:** CBF, blood pressure, tissue collection for 11,12-EET and PGE<sub>2</sub> ELISA.

### **3.4. Experiment 3:** Validation in aged, experienced Long-Evans rats

**Subjects:** 25 male Long-Evans rats (27.5 months old, 270-490 g), with >2 years cognitive training experience

**Treatment:** Initially 5/7/0.7 mg/kg MS-PPOH/L-NAME/indomethacin. Due to toxicity, reduced to 2.5/5/0.5 mg/kg in 20 % HPβCD + 1 % NaHCO<sub>3</sub>. Dosing volume 2 ml/kg, twice daily for 14 days.

#### **Cognitive assays:**

- Morris water maze (Days 5/6, 13)
- 5CSRTT (Days 2, 8, 13)
- Pot jumping (Days 5/6, 10)
- Touchscreen pairwise visual discrimination (Days 4, 9, 12)
- Cooperation task (Days 3, 7, 11)
- Y-maze alternation (Day 4, 14)

**Measurements:** CBF, blood pressure (Days 1, 3, 6, 9, 12), PGE<sub>2</sub> and 11,12-EET ELISA, histological analysis of kidney and brain.

### **3.5. Experiment 4:** Isolation of MS-PPOH effects

**Subjects:** 16 male Long-Evans rats (6.5 months old, 380-460 g)

**Treatment:** 5 mg/kg MS-PPOH alone, twice daily for 9 days, dissolved in 20 % HPβCD.

**Measurements:** CBF, Y-maze, blood pressure, 11,12-EET levels, kidney histology.

### **3.6. Experiment 5: Conditioned taste aversion (CTA)**

**Subjects:** 11 aged male Hannover Wistar rats (27-27.5 months, 410-550 g)

**Procedure:** 20-hour water deprivation, baseline consumption measurement, conditioning saccharin consumption with cocktail (5/10/1 mg/kg MS-PPOH/L-NAME/indomethacin) or vehicle treatment, followed by two-bottle choice tests for saccharin preference.

### **3.7. Statistical Analysis**

Repeated measures ANOVA, post-hoc tests, unpaired t-test, Mann-Whitney U-test. Significance at  $p < 0.05$ .

## **4. Results**

### **4.1. Experiment 1**

The treatment caused 54% inhibition of induced hyperaemia ( $p < 0.05$ ) and significantly elevated blood pressure (Treatment:  $F(1,25) = 7.405$ ,  $p < 0.05$ ).  $PGE_2$  levels were significantly reduced in barrel cortex ( $t(22)=7.272$ ,  $p < 0.001$ ) and small intestine ( $t(22)=2.888$ ,  $p < 0.01$ ). No cognitive impairments were observed. Intestinal alterations occurred in 8/14 rats, and one animal died.

### **4.2. Experiment 2**

No significant difference was observed in CBF, blood pressure, EET levels, or Y-maze performance. Cocktail-treated group showed significantly lower heart rate ( $F(1,15)=13.24$ ,  $p < 0.005$ ). Lever press and NOR assays produced insufficient evaluable data. The dose reduction eliminated toxicity but also NVU effects.

### 4.3. Experiment 3

The cocktail caused non-significant 28 % CBF reduction. Significant weight loss occurred in treated animals (7.8 % vs 2.8 %,  $t(23)=-3.066$ ,  $p < 0.01$ ) due to decreased food-intake. PGE<sub>2</sub> levels were significantly lower in brain ( $t(22)=2.954$ ,  $p < 0.01$ ) and small intestine ( $t(17)=-3.991$ ,  $p < 0.001$ ) while EET levels remained unchanged.

Cognitive effects were limited to 5CSRTT: decreased correct responses ( $F(1,23)=6.947$ ,  $p < 0.05$ ), increased omissions ( $F(1,23)=4.2524$ ,  $p=0.0507$ ), and reduced accuracy ( $F(1,23)=7.242$ ,  $p < 0.05$ ) on Day 8. No other cognitive task showed significant group differences. Histology revealed severe nephrotoxicity in treated animals (mean scores:  $10.5 \pm 0.96$  vs  $1.7 \pm 0.36$ ,  $p < 0.001$ ).

### 4.4. Experiment 4

MS-PPOH alone showed no significant effects on CBF, Y-maze performance, blood pressure, or brain EET levels. Kidney EET levels were significantly different ( $t(13)=2.849$ ,  $p < 0.05$ ). No notable histological alterations in kidneys.

### 4.5. Experiment 5

Cocktail-treated animals showed significantly reduced saccharin preference (Day 5:  $t(9)=4.219$ ,  $p < 0.01$ ; Day 6:  $t(9)=3.813$ ,  $p < 0.01$ ) and increased food leftovers (Day 6:  $t(9)=-3.406$ ,  $p < 0.01$ ) indicating cocktail-induced gastrointestinal distress.

*Table 1. Summary of the performed experiments. Each experiment is indicated with the number) of rats (N) involved. Apart from Experiment 5, all treatment consisted of two injections per treatment day (b.i.d.). In the case of Experiment 5, only 2 days involved 'cocktail-treatment'. In Experiment 3, because of toxic side-effects, starting dosages had to be adjusted. Final amounts are indicated with bolded numbers. Performed*

*cognitive task abbreviations: MWM: Morris water maze, 5C: 5CSRTT, PJ: pot jumping test, TS: touchscreen pairwise visual discrimination, Coop: cooperation task, YM: Y-maze alternation test, FC: fear conditioning, LP: lever-press learning, NOR: novel object recognition, CBF: cerebral blood flow, 5CSRTT: 5-choice serial reaction time task, CTA: conditioned taste aversion. \*: single dose*

| Study           | Subjects                       | Days  | Dosage (mg/kg, b.i.d.) |        |              | Cognitive tasks                   | Key Findings      |                         |
|-----------------|--------------------------------|-------|------------------------|--------|--------------|-----------------------------------|-------------------|-------------------------|
|                 |                                |       | MS-PPOH                | L-NAME | Indomethacin |                                   |                   |                         |
| Exp 1<br>(N=28) | Naïve, young – Wistar          | 8     | 5                      | 10     | 1            | FC, MWM                           | 54% CBF reduction |                         |
|                 |                                |       |                        |        |              |                                   | Severe toxicity   |                         |
| Exp 2<br>(N=17) | Naïve, young – Wistar          | 8     | 2.5                    | 5      | .5           | YM, LP, NOR                       | No CBF effect     |                         |
|                 |                                |       |                        |        |              |                                   | No efficacy       |                         |
| Exp 3<br>(N=25) | Experienced, aged – Long-Evans | 14    | 5<br>2.5               | 7<br>5 | .7<br>.5     | MWM, 5C<br>PJ, TS,<br>COOP,<br>YM | 28% CBF           | Transient 5CSRTT effect |
|                 |                                |       |                        |        |              |                                   | Toxicity          | MS-PPOH ineffective     |
| Exp 4<br>(N=15) | Naïve, young – Long-Evans      | 8     | 5                      | -      | -            | YM                                | No MS-PPOH effect |                         |
| Exp 5<br>(N=11) | Naïve, aged – Wistar           | 6 (2) | 5*                     | 10*    | 1*           | -                                 | CTA confirmed     |                         |

## 5. Conclusions

The primary objective of this doctoral work was to develop a pharmacologically induced NVU model in rats that could serve as a translational platform for modelling human cognitive decline. While previous work in mice showed promising results, translation to rats proved significantly more challenging than anticipated, revealing fundamental issues with the approach.

**Dose Optimization Reveals a Narrow Therapeutic Window:** Our initial experiment successfully induced NVU at reduced doses (54 % CBF reduction) but with substantial toxicity including elevated blood pressure, intestinal pathology, and mortality. Halving all doses eliminated both toxicity and efficacy, demonstrating a narrow therapeutic window. Even intermediate doses in aged animals caused severe age-dependent toxicity requiring continuous adjustments. This highlights that simple dose adjustment is insufficient to circumvent the trade-off between central efficacy and peripheral safety.

**Target Engagement Confirmation is Essential:** PGE<sub>2</sub> reduction confirmed successful COX inhibition in Experiments 1 and 3. However, EET levels remained unchanged across all experiments where measured, suggesting MS-PPOH was ineffective at the doses used; possibly due to poor blood-brain penetration. This underscores a critical gap: without establishing individual dose-response relationships for each compound measuring EET, PGE<sub>2</sub>, and NO levels in brain tissue, the contribution of each component to the overall NVU effect remains unknown. This foundational work must precede any combination therapy approaches.

**Cognitive Effects are Limited and Transient:** Only 5CSRTT showed significant impairment (transient attentional deficits on Day 8). The elevated omission rate likely reflects reduced motivation due to treatment-induced malaise, confirmed by the conditioned taste aversion results, while diminished accuracy suggests a genuine attentional deficit.

The transient nature may reflect subject resilience or compensatory mechanisms (e.g., upregulated enzyme synthesis) that develop to counteract pharmacological inhibition over time.

**Age-Dependent Toxicity is a Critical Limitation:** Aged animals showed dramatically reduced food intake, significant weight loss, gastrointestinal distress (confirmed by CTA), and severe nephrotoxicity—effects not observed in young animals. This represents a major limitation, particularly as aged populations are the primary target for cognitive enhancers, where such side effects may substantially confound behavioural and physiological assessments.

**Methodological Challenges Require Advanced Approaches:** High inter-subject variability in CBF measurements necessitates large sample sizes or self-controlled designs. Measuring NVC in cognitively relevant regions like the hippocampus demands more sophisticated approaches. Developing chronic, non-invasive NVU assessment methodologies (such as fMRI or fUS) would enable longitudinal studies and address variability concerns.

### **Path Forward for Model Refinement:**

1. **Establish individual dose-response relationships** for each compound measuring EET, PGE<sub>2</sub>, and NO levels in brain and peripheral tissues.

2. **Explore alternative administration routes** such as intracerebroventricular delivery to circumvent peripheral toxicity and blood-brain penetration issues, though formulation challenges must be addressed.
3. **Develop chronic, non-invasive NVU assessment** using fMRI or fUS to enable longitudinal tracking of induced NVU without terminal procedures.
4. **Investigate compensatory mechanisms** that may obscure NVU effects and contribute to transient cognitive impairments.
5. **Assess drug synergy and age sensitivity** systematically, as combined compounds may have synergistic effects not predicted from individual profiles.

**Overall Feasibility and Translational Value:** Model development is vital for pharmaceutical research to ensure smooth transition from preclinical results to clinical success. While the published mouse study suggested transferring this pharmacological NVU model to rats would yield a straightforward method for drug screening, our findings illustrate that achieving this goal is far more complex, demanding significant technological resources with uncertain feasibility. However, securing funds for developing such models should be considered of utmost importance, because decisions based on inadequately validated models are a primary cause of late-stage clinical failures.

Our work has identified substantial barriers to reliable pharmacological NVU induction in rats while simultaneously outlining a clear roadmap for model refinement. Successfully addressing these challenges through the recommended approaches will be crucial for creating robust preclinical models that can effectively bridge the gap to clinical applications in cerebrovascular cognitive impairment research.

## **6. Bibliography of the candidate's publications**

### **Own publications involved in the current thesis**

1. Varga BT, Ernyey AJ, Tajti BT, Gáspár A, Demeter Z, Kollár L, et al. Translational difficulties in establishing a pharmacologically induced neurovascular uncoupling model in rats. 2026. doi: 10.1038/s41598-026-58244-0 In press
2. Varga BT, Gáspár A, Ernyey AJ, Hutka B, Tajti BT, Zádori ZS, et al. Introduction of a pharmacological neurovascular uncoupling model in rats based on results of mice. *Physiol Int.* 2022 Sep 5;109(3):405–18. doi:10.1556/2060.2022.00226 PubMed PMID: 36057105.

#### **Conference papers:**

1. Varga BT, Ernyey AJ, Demeter Z, Tajti BT, Gáspár, A, Zádori ZS, et al. Pharmacologically-induced neurovascular uncoupling in rats: model development challenges and insights. *Neuroscience Applied* 5: 1 pp. 106061---. Paper: 106061 (2026)
2. Varga BT, Ernyey AJ, Demeter Z, Tajti BT, Gáspár, A, Zádori ZS, et al. Challenges and lessons in developing a pharmacologically-induced

neurovascular uncoupling model in rats. International Pharmacology Conference: focus on drug discovery and innovation (HUPHAR 2025): Oral & Poster Abstracts (2025) 226 p. pp. 181-181. Paper: 43, 1 p.

### **Own publications not involved in the current thesis**

1. Al-Khrasani M, Essmat N, Boldizsár I, Varga BT, Chalabiani Y, Abbood SK, et al. Do vitamins halt the COVID-19-evoked pro-inflammatory cytokines involved in the development of neuropathic pain? *Biomedicine and Pharmacotherapy*. 2025;189.
2. Essmat N, Boldizsár I, Chalabiani Y, Varga BT, Abbood SK, Kirchlechner-Farkas JM, et al. Crosstalk Between Glycinergic and N-Methyl-D-Aspartate Receptor-Mediated Glutamatergic Transmission in Behaviours Associated with Opioid Use Disorder. *International Journal of Molecular Sciences*. 2025;26(21):10526. doi:10.3390/ijms262110526
3. Tajti BT, Yoon O, Ernyey AJ, Gáspár A, Varga BT, Gyertyán I. Using Appetitive Motivation to Train Mice for Spatial Learning in the Barnes Maze. *Biomed Research International*. 2023;2023.
4. Gáspár A, Hutka B, Ernyey AJ, Tajti BT, Varga BT, Zádori ZS, et al. Intracerebroventricularly Injected Streptozotocin Exerts Subtle Effects on the Cognitive Performance of Long-Evans Rats. *Frontiers in Pharmacology*. 2021;12. doi:10.3389/fphar.2021.662173  
Corrigendum: Intracerebroventricularly Injected Streptozotocin Exerts Subtle Effects on the Cognitive Performance of Long-Evans Rats (*Front. Pharmacol.*, (2021), 12, (662173), 10.3389/fphar.2021.662173). *Frontiers in Pharmacology*. 2023;13.
5. Gáspár A, Hutka B, Ernyey AJ, Tajti BT, Varga BT, Zádori ZS, et al. Performance of the intracerebroventricularly injected streptozotocin

Alzheimer's disease model in a translationally relevant, aged and experienced rat population. *Scientific Reports*. 2022;12(1).

Conference papers:

1. Ernyey AJ, Gáspár A, Kassai F, Hidasi F, Tajti BT, Varga BT, et al. Rats use random strategy for solving complicated cognitive tasks. Conference on Pharmacology, Pharmacokinetics & Innovation (HUPHAR 2024): Oral & Poster Abstracts (2024) 217 p. pp. 179-180., 2 p.
2. Gáspár A, Ernyey AJ, Hutka B, Tajti BT, Varga BT, Zádori ZS et al. Cognitive performance of aged, experienced Long-Evans rats after icv streptozotocin treatment. *European Neuropsychopharmacology* 53: Suppl. 1 pp. S620-S621. Paper: P.0848, 2 p. (2021)
3. Gyertyán I, Kassai F, Gáspár A, Varga BT, Ernyey AJ P.0549 A complex experimental design for measuring the learning impairing effect of repeat dose scopolamine in experienced aged rats. *European Neuropsychopharmacology* 53: Supplement 1 pp. S404-S404. (2021)
4. Varga, BT, Ernyey AJ, Tajti, BT, Gáspár A, Gyertyán I. P.0849 Freezing and ultrasound vocalization in response to contextual and discrete conditional stimuli in a fear conditioning paradigm *European Neuropsychopharmacology* 53: Supplement 1 pp. S621-S622. (2021)
5. Gáspár, A, Ernyey AJ, Hutka B, Tajti BT, Varga BT, Zádori Z et al. P.376 Investigating the effect of intracerebroventricularly injected streptozotocin in various learning paradigms in Long-Evans rats. *European Neuropsychopharmacology* 40: Suppl. 1 pp. S216-S217. (2020)