Photocontrollable probes for the investigation of neuropeptide signaling

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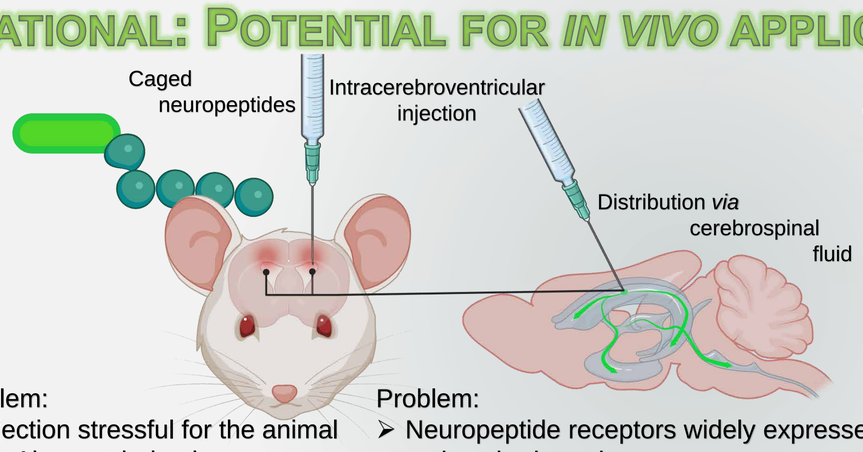
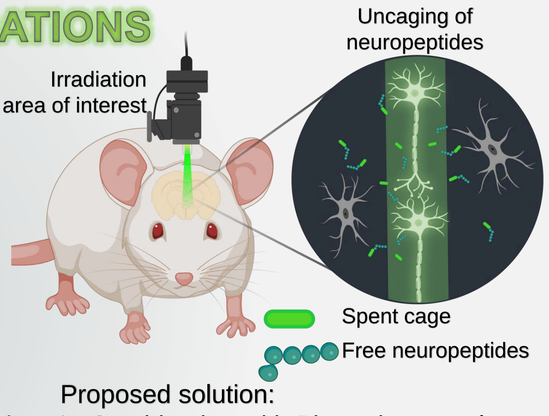
The Oxytocin/Vasopressin System and Photopharmacology

- Oxytocin/Vasopressin (OT/VP) are highly conserved neuropeptides playing a crucial role in physiological and neurological functions, including emotional responses and learning.[1-3]
- Dysfunctions in the OT/VP system have been linked to pathophysiological and psychological conditions, such as breast cancer, autism spectrum disorder, and major depressive disorder.
- Underpinning neuropeptide-mediated pathways have not been fully elucidated[1,3,4]
- Receptors widely expressed throughout the brain making it difficult to investigate specific effects of neuropeptides *via* intracerebroventricular or stereotaxic injections.[5]
- To address this complexity, we developed advanced photoprobes for OT and VP, enabling precise spatiotemporal control over neuropeptide activity *via* photopharmacology.
- Photolabile protecting groups (photocages) mask the peptides' bioactivity until exposed to light (1PE/2PE), allowing controlled release of OT and VP.
- The effectiveness of these probes was validated in cellular assays and *in vitro* experiments, offering a powerful tool for future studies on neuropeptide signaling in the brain
- Applications of our probes in prospective *ex vivo* and *in vivo* studies could potentially advance the understanding of neuropeptide related physiological and pathological conditions.

Rational: Potential for *in vivo* applications



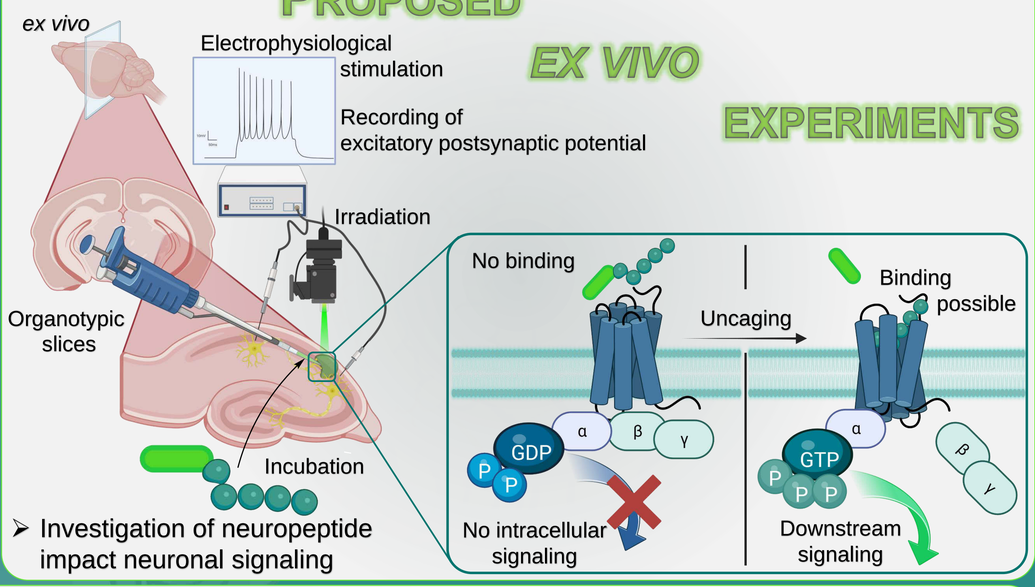


Problem:
- Injection stressful for the animal → Aberrant behaviour
- Usually anaesthesia required → Limited time window for behavioural experiments

Problem:
- Neuropeptide receptors widely expressed in various brain regions
- Diffusion away from original point of injection
- Interactions in non-targeted areas → Complicates evaluation of behavioural output in correlation to specific brain areas

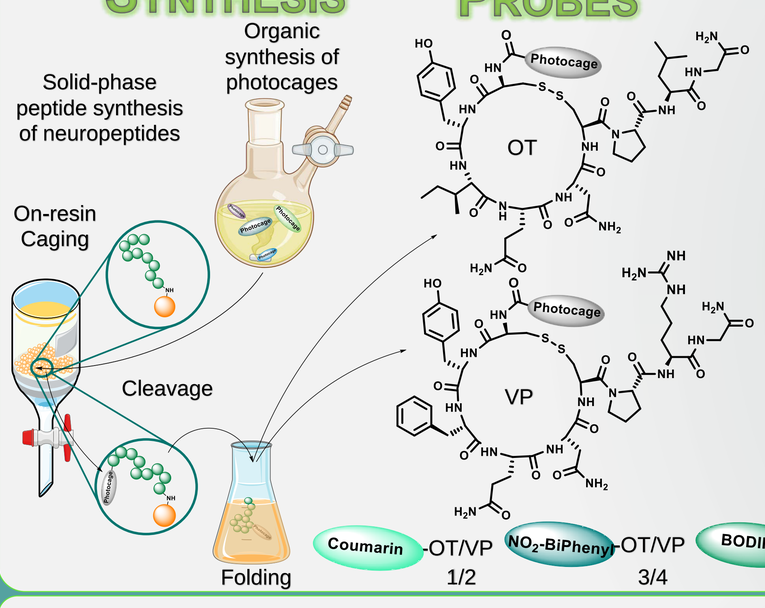
Proposed solution:
- Combination with Photopharmacology → Photocaging
- → High spatiotemporal control
- → Uncaging during *in vivo* training
- → Simulation of endogenous release

Proposed *ex vivo* experiments



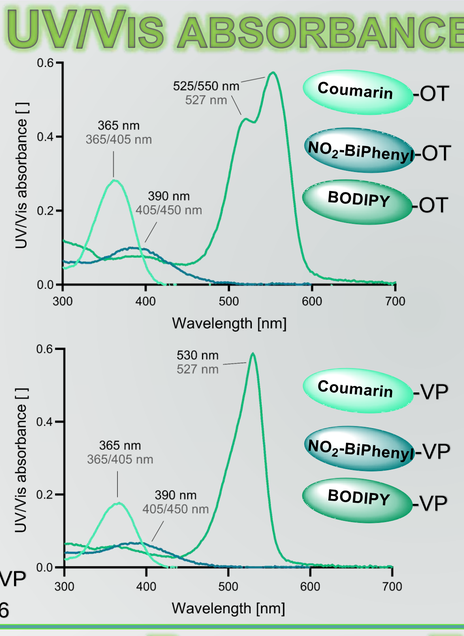
- Investigation of neuropeptide impact neuronal signaling

Synthesis

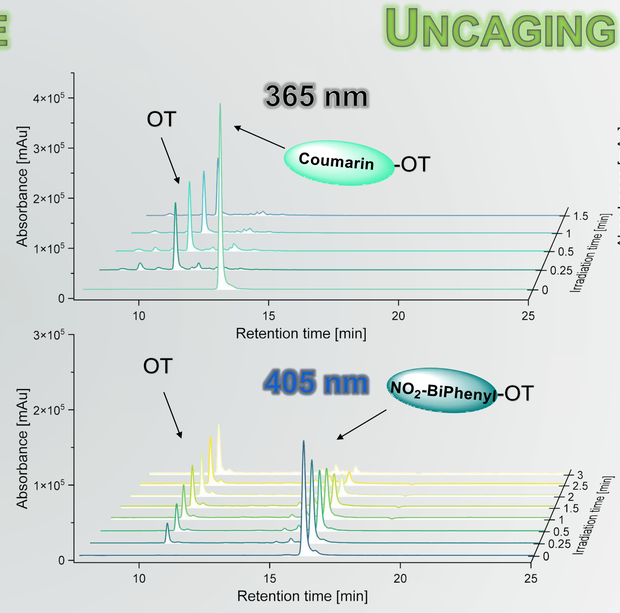


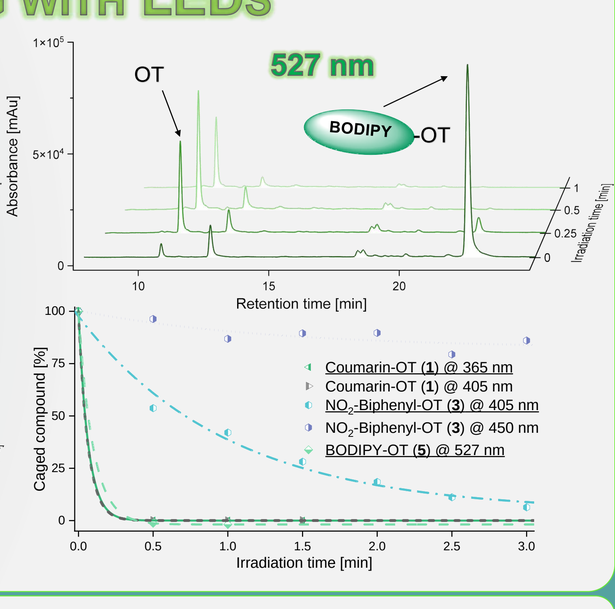
Probes

UV/Vis absorbance



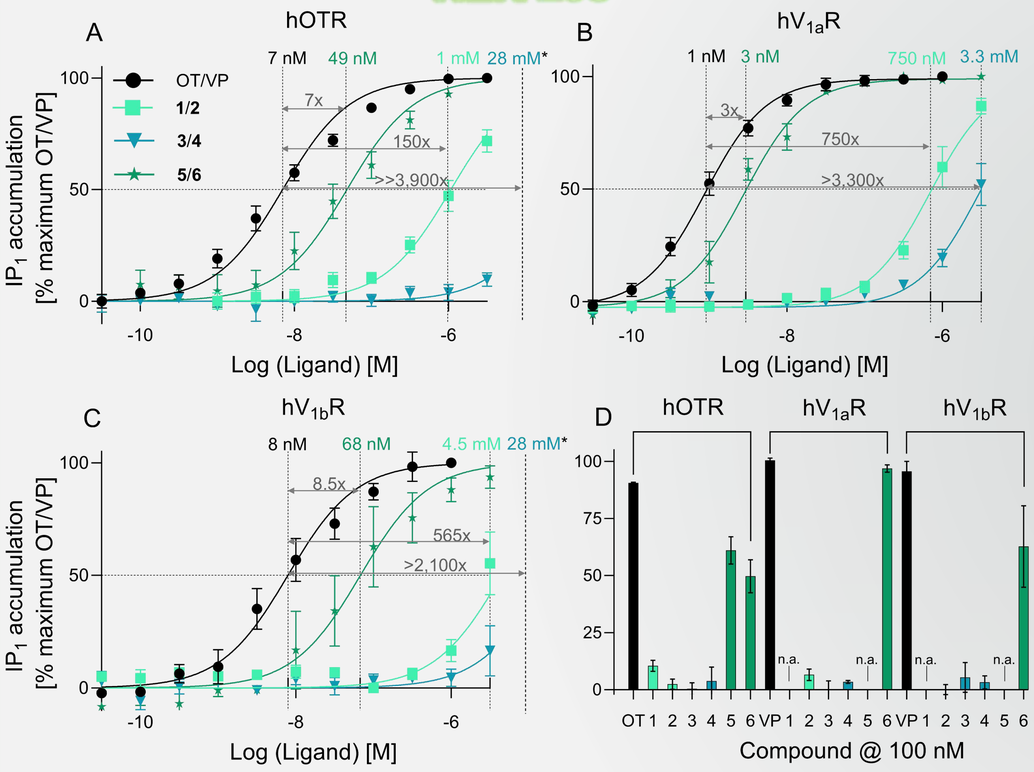
Uncaging with LEDs



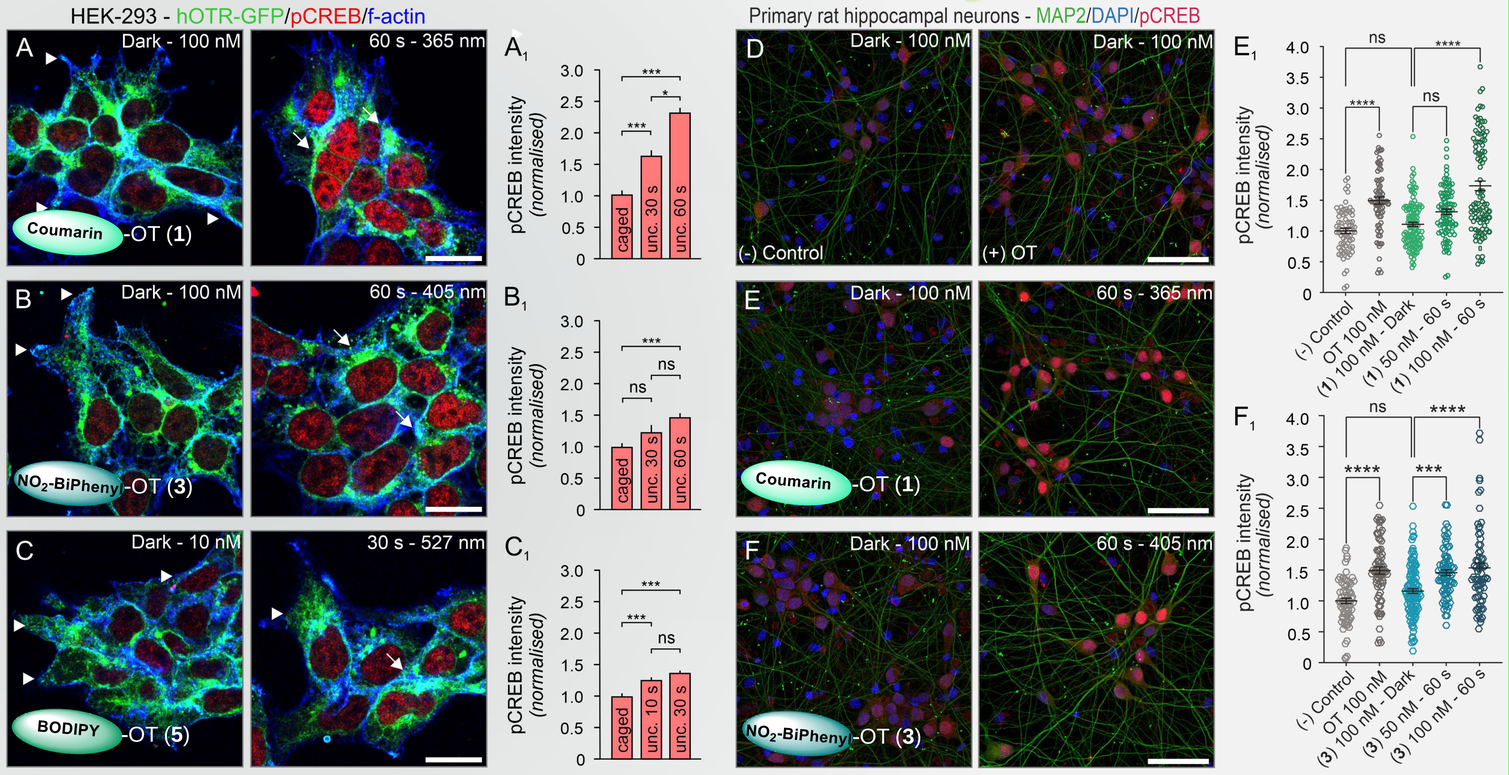


Biological Evaluation

Functional IP_1 Assays HEK-293



Confocal Scanning Microscopy Immunostaining & Quantification of pCREB



In vitro pharmacologically evaluation of photocaged neuropeptides. Cellular functional IP_1-assay performed on stable HEK-293 cells, overexpressing hOTR, hV1aR, or $hV_{1b}R$. Each point represents at least three independent measurements in technical triplicates. Results were normalised to OT/VP (100%) and negative control (buffer; 0%) activity. Error bars indicate the standard error of the mean. (A) hOTR, (B) $hV_{1a}R$, (C) $hV_{1b}R$, (D) 100 nM at each receptor. EC_{50} values shown above with same color coding for the corresponding compounds, asterisks indicate extrapolated values, grey numbers and lines indicate x-fold increase of EC_{50} values compared to parent peptide, n. a. no activity at 100 nM.

In vitro evaluation of the biological activity of OT photoprobes caged and uncaged. Left HEK-293 cells overexpressing hOTR-GFP. Phosphorylation of CREB (red) quantified after immunocytochemistry normalised to caged treatment. (A, A_1) Coumarin-OT (**1**), 100 nM, irradiation time 30 s and 60 s at 365 nm. (B, B_1) NO_2-Biphenyl-OT (**3**), 100 nM, irradiation time 30 s, 60 s at 405 nm. (C, C_1) BODIPY-OT (**5**), 10 nM, irradiation time 10 s, 30 s at 527 nm. Note transfer of hOTR-GFP signals (green) from membrane (blue, arrowheads) into cytosol (arrows), indicating receptor internalisation after uncaging. ns, non-significant; *P<0.05; ***P<0.001. Scale bars = 15 µm. Right primary rat hippocampal neurons. Phosphorylation of CREB (red) quantified after immunocytochemistry and normalised to negative control (aCSF). (D) Negative control (aCSF) and positive control (OT, 100 nM) dark, (E, E_1) Coumarin-OT (**1**), 100 nM, dark and irradiated at 365 nm for 60 s. (F, F_1) NO_2-Biphenyl-OT (**3**), 100 nM, dark and irradiated at 405 nm for 60 s. ns, non-significant; ***P<0.0005; ****P<0.0001. Scale bars = 50 µm.

Results

- Synthesis and comparison of three different photocages
- Reduced activities of caged compounds compared to parent peptides (up to >>3,900-fold)
- Fast and robust uncaging with easy-to-implement LED setup
- Uncaging *in vitro* possible and compatible with neuronal cells
- Irradiation induces cellular response comparable to endogenous peptides

Outlook

- Two-photon uncaging experiments
- Electrophysiology *ex vivo* experiments in organotypic tissue slices
- Selection of most promising compound for prospective *in vivo* experiments
- Extension to other important neuropeptides (Somatostatin, α-MSH, etc.)
- Manuscript in preparation

References

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Legend

1PE: one-photon excitation; 2PE: two-photon excitation; aCSF: artificial cerebrospinal fluid
pCREB: phosphorylated cAMP response binding protein; GFP: Green fluorescent protein

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