Optofluidic control of rodent learning using cloaked caged glutamate

Romain Durand-de Cuttoli, Pradeep S Chauhan, Adriana Petriz Reyes, Philippe Faure, Alexandre Mourot, Graham Ellis-Davies

Optofluidic control of rodent learning using cloaked caged glutamate.

Romain Durand-de Cuttoli[1,3], Pradeep S. Chauhan[2,3], Adriana Pétriz Reyes[2,3], Philippe Faure[1], Alexandre Mourot[1]* and Graham C.R. Ellis-Davies[2]*.

[1]Neuroscience Paris Seine, Institute de Biologie Paris Seine, Sorbonne Université, Paris, France.

[2]Department of Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA.

[3]Equal contribution.

correspondence: almourot@gmail.com or graham.davies@mssm.edu.

Key words: uncaging, optofluidics, GABA-A antagonism, biologically inert, conditioned place-preference.

Abstract.

Glutamate is the major excitatory neurotransmitter in the brain, and photochemical release of glutamate (or uncaging) is a chemical technique widely used by biologists to interrogate its physiology. A basic prerequisite of these optical probes is bio-inertness before photolysis. However, all caged glutamates are known to have strong antagonism towards receptors of the γ-aminobutyric acid, the major inhibitory transmitter. We have developed the first caged glutamate probe that is inert towards these receptors at concentrations that are effective for photolysis with violet light. Pharmacological tests *in vitro* revealed that attachment of a fifth-generation (G5) dendrimer to the widely used 4-methoxy-7-nitro-indolinyl(MNI)-Glu probe (i.e. "cloaking") prevented such off-target effects, whilst not changing the photochemical properties of MNI-Glu significantly. G5-MNI-Glu was used with "optofluidic" delivery to stimulate dopamine neurons of the ventral tegmental area of freely-moving mice in a conditioned place-preference protocol so as to mediate Pavlovian conditioning.

Significance statement.

Caged glutamates are photolabile compounds that are widely used by neuroscientists. However these probes have off-target pharmacological side effects in that they block inhibitory neurotransmitter receptors. We develop a new caged glutamate molecule decorated with a large "dendrimer cloak" that prevents such blockade. This photoprobe is the first example of a caged glutamate that is fully biologically inert. We combine this compound with the newly developed technique of "optofluidics". This method allows us deliver the probe with simultaneous photolysis in freely moving mice. Uncaging in the brain region involved in the reward pathway mediated Pavlovian conditioning during a behavioral test. This work forms useful paradigm for future experiments involving real-time phasic manipulation of the behavior of higher order animals

Photochemical release of chemically protected biomolecules has been very widely used for control of cell signaling *in vitro* for over 40 years(1-7). The reason for this is that optical and chemical technologies form a powerful pair to enable biological experimentation. Well-defined structure-activity relationships of biomolecules allow synthetic organic chemists to create biologically inert probes that respond to light delivery(1). Optical technology can deliver light to broad areas to envelope many cells, or be used to focus and direct illumination to subcellular locations. Thus, molecules such as ATP, cAMP, IP_3 and Ca^{2+} have all been released inside cells, and neurotransmitters on cells, to enable many hundreds of studies of cellular physiology and biochemistry in cultured cells(1, 8).

In contrast, the delivery of light with chemical probes *in vivo* has proved challenging, thus limiting the use of photochemically responsive organic molecules in freely moving animals. Examples of uncaging ATP in drosophila(9) or photoswitching glutamate receptors in zebrafish(10) take advantage of the optical transparency of such species. However, application of this approach to rodents have been restricted mainly to head-fixed animals(11-13). Recently, we have developed an "optofluidic" approach for control of rodent behavior with a synthetic photoswitch that circumvents many such limitations(14). Furthermore, other studies have described fabrication of novel head-mounted(15) or tethered optofluidic(16) systems for drug and light delivery in freely moving mice.

Glutamate uncaging has been used by neurophysiologists from when it was first realized in complex brain tissue in 1993(17). Since the first set of caged glutamate probes were developed by biochemists(18, 19), the idea that such molecules might have off-target interactions with other receptors such as those for γ-aminobutyric acid (GABA)-A was not examined. Indeed the first examples of "second generation" nitroindolinyl-caged glutamate probes were reported to be non-antagonistic towards GABA-A receptors(20). Thus, it came as a real surprise that probes such as 4-methoxy-7-nitro-indolinyl(MNI)-Glu had such off-target antagonism(21). But since caged glutamates do not antagonise ionotropic glutamate receptors (20, 22) the off-target effects do not limit

the use of such probes for the study of these receptors i*n vitro*(23). However, when MNI-Glu was used *in vivo* we found that co-application of the Na channel blocker tetrodotoxin (TTX) was required in order to block undesirable side effects from GABA-A receptor antagonism(13). While the idea of reducing antagonism of caged neurotransmitters by decoration with a high density of negative charge seems very reasonable(24), in practice it has been found the improvements are, at best, modest(25). Indeed one negative charge is as (in)effective as four(26-28). These data suggested to us that a completely novel means of reducing GABA-A antagonism was required. So in 2017 we advanced the idea of "enveloping" the caged neurotransmitter with a "dendrimer cloak" to prevent probe binding to GABA-A receptors. Thus, a cloaked caged GABA was found to have approximately 90-fold lower antagonism than its non-cloaked analogue(29). Here we describe the application of cloaking technology to *caged glutamate*. We show that at concentrations that are very effective for one-photon (1P) photolysis, a cloaked caged glutamate (**1**) is essentially inert, and can be used for photorelease of glutamate *in vitro* and *in vivo*. Importantly, we show that the new probe can be used with an optofludic device(14) to control rodent learning in a conditioned place preference protocol.

Results.

The synthesis of **1** started with **2**(30), using the pendant carboxylate to attach an azide functionality via condensation aminopropylchloride to give **3**, followed by halogen replacement by reaction with sodium azide, to give **4** (Fig. 1). The nitro functionality was installed by reaction of **4** with Claycop to give fully protected caged glutamate **5**. This intermediate was then conjugated with the dendrimer. A 5th generation (G5) polyester dendron with an alkyne focal point and a neutral hydroxyl periphery was used for copper(I)-catalyzed azide-alkyne "click" cycloaddition to give fully protected G5-MNI-Glu **6**, which could be purified using flash chromatography on silica gel. Brief treatment of **6** with trifluoacetic acid yielded the target cloaked caged glutamate compound, **1**, in essentially quantitative yield. Importantly, and unlike MNI-Glu, which requires HPLC

for use with brain slices(22), this compound can be applied to slices for hours without any apparent toxic effects towards neurons (see below).

Photolysis of 7-nitroindolinyl-protected acids has been well characterized, and is known to give the uncaged acid and nitrosoindole in essentially quantitative yields(31-34). The change in UV-visible absorption spectrum of the aromatic chromophore correlates quantitatively with acid release(32), so can be used to follow the uncaging reaction and thus measure the quantum yield of photolysis. Therefore, when individual solutions of either MNI-Glu or G5-MNI-Glu were irradiated at the reaction isosbestic point using 365-nm light, the time-course of the change of the latter occurred at a rate that was 87% of the former (Fig. 1, box), corresponding to a quantum yield of photolysis of 0.057 (using the MNI-Glu quantum yield of 0.065(35)). Encouraged by these results, we tested the physiological efficacy of G5-MNI-Glu *in vitro* on neurons in acutely isolated brain slices.

Pyramidal neurons from the CA1 region of the hippocampus were filled with a fluorescent dye via a patch pipette, and imaged using 2-photon microscopy (Fig. 2a). This allowed us to direct a violet laser (410 nm) to the edge of the cell body using galvanometer control. Local perfusion of a solution of G5-MNI-Glu (1 mM) from a pipette position just above the surface of the brain slice delivered the caged compound to the selected cell. Irradiation produced robust inward currents from three points that were highly reproducible in size (Fig. 2b). These currents are similar in size to those evoked by photolysis of MNI-Glu (bath applied at 0.66 mM) on the same microscope in a different study(36). Similar results were detected when voltages were recorded (Fig. 2c). Next we tested the lateral resolution from 1P laser uncaging on cells. When the laser was moved in 1 μm increments away from the cell body, similar to previous reports(21), the signals on single trails were barely detectable above noise at a distance of 4-5 μm (Fig. 2d,e). Cellular responses were found to be graded with laser power, in a linear manner, consistent with 1P uncaging(36, 37) (Fig. 2f,g). In a final set of experiments using G5-MNI-Glu with CA1 neurons we established uncaging at single spines performed in a similar manner to MNI-Glu (SI Appendix Fig. S1). To prepare for use of

G5-MNI-Glu *in vivo*, we also tested uncaging on midbrain dopamine neurons using full illumination with light from a 385-nm LED. For these experiments the patch-clamped cell was positioned in the middle of the field of view, and light was delivered through the epi-fluorescent port of the microscope. Photolysis again produced an inward current (Fig. 2h).

Having shown previously that the unsubstituted G5-OH dendrimer has no detectable antagonism in the mM range(29), we used the effect on miniature inhibitory postsynaptic currents (mIPSCs) as means to test the GABA-A receptor antagonism of G5-MNI-Glu. The probe was bath-applied to brain slices in four successively increasing concentrations (0.25, 0.50. 0.75 and 1.0 mM), followed by wash out of the recording chamber. The mIPSCs from CA1 neurons were recorded in the presence of TTX (to block action potentials, APV (to block NMDA receptors) and CNQX (to block non-NMDA receptors). Over the entire concentration range there was no significant reduction in the amplitude of the mIPSCs (Fig. 3a; student t-test; $P > 0.05$; n = 3 cells), suggesting no antagonism of GABA-A receptors. Overall there was no significant reduction in the frequency of the mIPSCs either (Fig. 3b; student t-test; $P > 0.05$; n = 3 cells), except at 0.25 mM there was a slight increase (Fig. 3c; student t-test; $P = 0.0098$; n = 3 cells). Fig. 3c shows representative recordings of mIPSCs at the beginning and end of one such experiment, with mIPSCs from the highest [G5-MNI-Glu] tested shown. These data suggested to us that G5-MNI-Glu could be useful for glutamate uncaging in experiments where the balance of excitation and inhibition is important, for example in freely behaving animals. Thus, we tested the new probe during Pavlovian conditioning, using "optofludic" uncaging in the ventral tegmental area (VTA), a midbrain region with dopaminergic nuclei critically involved in reward-related behaviors.

Stimulation of dopamine neurons in the VTA is critical for behavioral learning in conditioned place preference tests(38, 39). Classical methods such as electrical stimulation or infusion of drugs of abuse have been used to induce reinforcement. Thus, a simple conditioned place preference test is a very robust means to assay whether uncaging of glutamate can cause increase in firing of dopamine neurons *in vivo* so as to

encode learning or experience. Using the stereotaxic coordinates for the VTA (see Supplemental Information), we positioned unilaterally optofluidic devices on 11 mice. The cannulas were then lowered carefully to be above the VTA so as to allow drug and light delivery without mechanically disturbing the brain region itself (Fig. 4a). Two to three weeks after surgical implantation, mice were subjected to a conditioned place-preference protocol in which they were allowed to explore two chambers for a 900 s period on day 1 (pretest). Each environment is novel and neutral in this behavioral test, however the walls and floors are distinct so can be distinguished subsequently. On days 2-4 mice were placed on each side for 600 s, and **1** was infused continuously at 2.5 mM during these periods (pairing, i.e. the conditioning period). However, only on one side was light applied (390 nm, 10 mW, 200 ms, 2 Hz). Note we have previously shown that there is a 5-fold dilution of caged glutamate during uncaging experiments *in vivo*(13), implying that there was approximately 0.5 mM G5-MNI-Glu available for uncaging in the mouse brain. On day 5 (test), both chambers are available for exploration, and the residence of the mouse is recorded (Fig. 4c). For the pairing days, a light guide and tube were connected to the cannula (Fig. 4a), but not during the pretest and test days. The residence times for each mouse on the pretest day 1 was compared to the test day 5 (Fig. 4d). Overall, we found there was a significant increase in residency time in the chamber associated with uncaging compared to the other chamber for the 11 mice tested, indicating behavioral conditioning (Fig. 4d, $P > 0.05$, paired, non-parametric Wilcoxon rank test). Since G5-MNI-Glu is inert up to 1 mM (Fig. 3), and the neuronal response from uncaging is linear (Fig. 2), our data imply one could use a wide variety of energies, and concentrations to achieve similar effects.

Discussion.

With the recent introduction of single piece, highly flexible implantable optofluidic probes(16), or even more sophisticated entirely head-mounted devices for wireless photopharmacology(15), the need for photo-activatable drugs will increase for such devices to be used widely. Such probes will enable precise phasic delivery of drugs via optofluidics in a manner that complements the tonic delivery normally realized by slow

infusion into the rodent brain. To test the feasibility of development of such caged compounds for *in vivo* use we selected glutamate as the "drug" test bed. First, the pharmacological concentration demand for glutamate is very high, as the target receptors have very low affinities for the neurotransmitter (ca. 10-100 micromolar), and our new caged glutamate probe was effective in the 0.25-1.0 millimolar range (Fig. 2). Secondly, caged glutamate probes have well known off-target side effects(21, 40), providing scope for testing our new cloaking method for reduction of such. The cloaked caged compound, G5-MNI-Glu, showed no GABA-A receptor antagonism up to 1 mM (Fig. 3). Thirdly, in principle, uncaging glutamate allowed the new probe to be used in a well-defined behavioral assay, one that could be validated by comparison with other temporally well-defined methods. *In vivo* uncaging of glutamate on dopamine neurons in the VTA produced a robust behavioral effect (Fig. 4), one that equaled other well-established approaches(14, 39). Our data suggest that cloaked caged compounds might provide one general method for delivery of caged pro-drugs with optofludics. In particular, we could imagine caged receptor-specific antagonists using such technology. Often such drugs are quite lipophilic, and the addition of aromatic caging chromophores only exacerbate this problem. Another benefit of cloaking is that dendrimers are highly biocompatible and soluble in physiological buffer(41, 42). Photopharmacology is a topic of intense current interest(43-48), our work is the first example of using optofluidic delivery of a caged compound in freely moving rodents, and thus describes a useful paradigm for this type of experiment with future caged probes that will allow phasic manipulation of the behavior of higher order animals.

Methods.

Chemical synthesis and photochemistry.

See SI Appendix online for full synthetic procedures. The quantum yield for photolysis of **1** was measured by comparison with the rate of photolysis with MNI-Glu. The rate of change of absorption of 0.1 mM solutions of MNI-Glu and **1** in HEPES buffer (40 mM,

pH 7.4, 100 mM KCl) were measured in a 1-cm cuvette during photolysis with a 365-nm LED (Thorslabs, Newton, NJ, USA).

Physiology.

All animal experiments were approved and performed according institutional IACUC rules. Brain slices were prepared acutely from C57BL/6J mice as described in(49). Brain slices were transferred to the recording chamber and perfused with carbogenated artificial cerebral spinal fluid (ACSF) at RT. Whole-cell recordings were made from hippocampal CA1 pyramidal or midbrain dopamine neurons. Patch pipettes with a resistance of 3-5 MΩ were filled with different internal solutions. For uncaging experiments, cells were patch-clamped at -60 mV. For mIPSC recordings, cells were clamped at +10 mV and recorded in the presence of 1 μM TTX, 10 μM CNQX and 50 μM D-AP5 applied via the perfusion system, and analyzed in LabVIEW (National Instruments).

Laser uncaging on CA1 neurons was performed on an Olympus BX61 microscope fitted with a Prairie Technologies Ultima dual-galvo scan head and Vision II Ti:Sapphire laser, a continuous-wave 410-nm laser, and an EPC10 amplifier. Dopamine neurons were characterized in current clamp mode as described(14). Whole-cell recordings were performed using an Axoclamp 200B amplifier. LED uncaging used full field illumination through the epi-fluorescence pathway(36) with a 385-nm LED (100 ms, pE-2, CoolLED), with a light output of 6.5 mW, corresponding to 5 mW/mm^2 at the focal plane.

Mice (C57BL/6JRj) were implanted unilaterally with a chronic multiple-fluid/optical injector (Doric Lenses Inc., Canada) at coordinates: from bregma, in mm: AP = 3.1 – 3.3; ML = 0.5 – 0.6; DV = 4. The guides (length = 4 mm from skull surface, OD = 450 – 650 μm) combined a fluid injection needle (protruding to 4.5 mm from skull surface) for delivering G5-MNI-Glu and an optic fiber (200 μm core, NA=0.66, protruding to 4.3 mm from skull surface) for light delivery coupled to a ceramic ferrule (1.25 mm). Between experiments, a plug was used to close the cannula and seal the

implant. The implant was attached to the skull with dental cement (SuperBond, Sun Medical).

The conditioned place preference (CPP) apparatus used a Y-maze (Imetronic, France) with one closed arm, and two other arms with manually operated doors. Two rectangular chambers (11x25 cm), with different cues (texture and color), were separated by a central neutral triangular compartment (side of 11 cm). One pairing compartment had grey textured floor and walls and the other smooth black and white striped walls and floor. See SI Appendix online for a detailed description of the full physiological procedures. Data Availability Statement: All data discussed in the paper will be made available to readers.

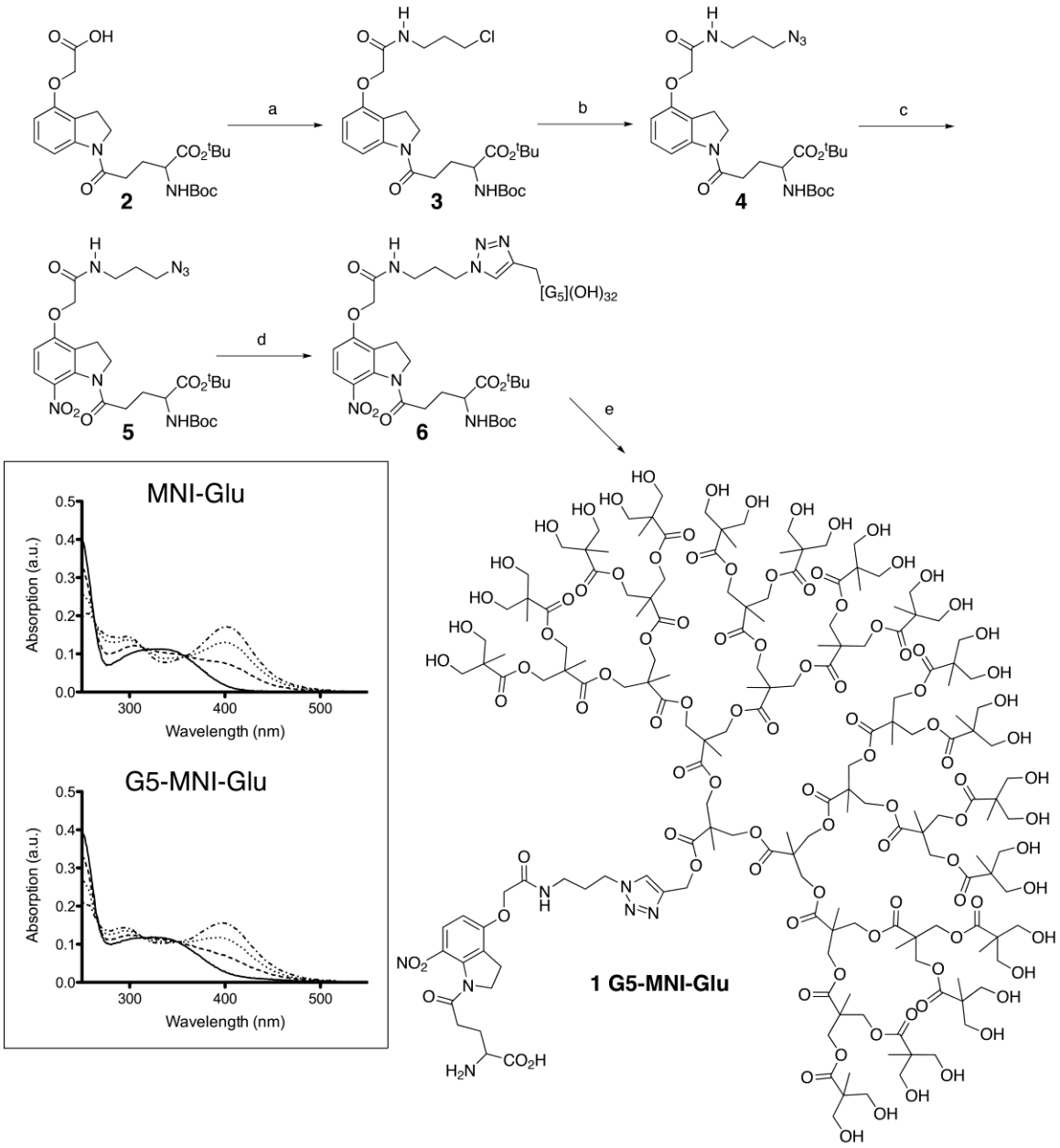


Figure 1. Synthesis and photochemistry of G5-MNI-Glu. Reagents and conditions: a) 3-chloropropylamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; b) NaN_3; c) Claycop, $(AcO)_2O$; d) $Cu(II)SO_4$, Na ascorbate; e) Trifluoroacetic acid. Box: change in absorption spectra of MNI- and G5-MNI-Glu when irradiated at 365 nm.

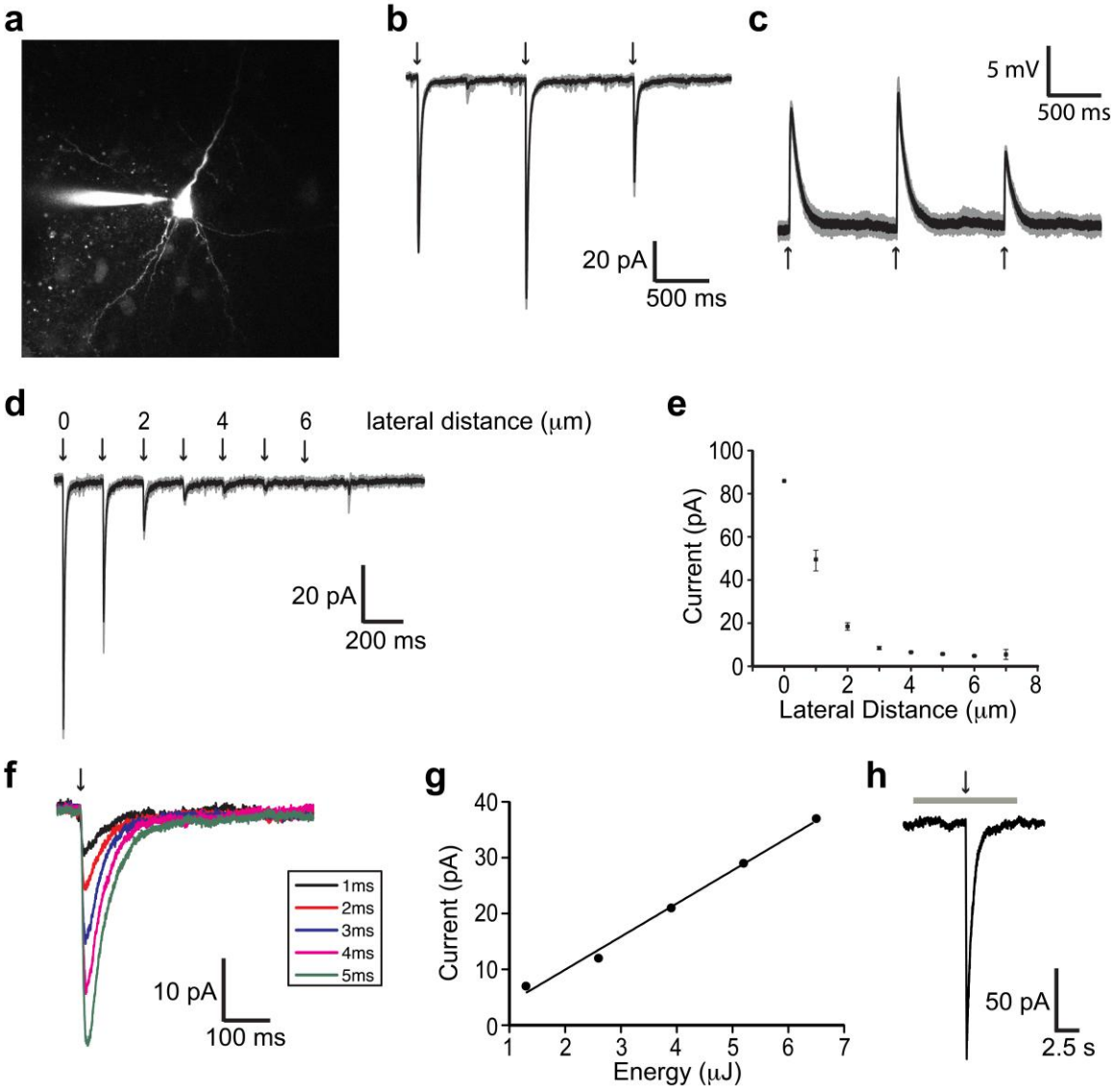


Figure 2. Uncaging on cells *in vitro*.

G5-MNI-Glu was locally perfused onto neurons in acutely isolated brain slices from glass pipettes positioned just above the slice surface. Arrows indicate the timing of using a 410 nm laser and 1.3 mW (a-g), or 385-nm LED (h). (a) Two-photon fluorescent image of a patch-clamped CA1 neuron filled with Alex594. (b) Currents evoked from CA1 neurons from laser irradiation (5 ms) at 3 points around the soma. (c) Changes in membrane potential evoked under the same conditions as (b). (d,e) Currents evoked by moving the laser focus laterally with 1-micron steps. (f) Photo-evoked currents produced by increasing the uncaging period. (g) Evoked current increased linearly with energy (r^2

= 0.99). (h) Current evoked by photolysis (full field LED illumination) on a VTA dopamine neuron. The grey bar illustrates the perfusion period.

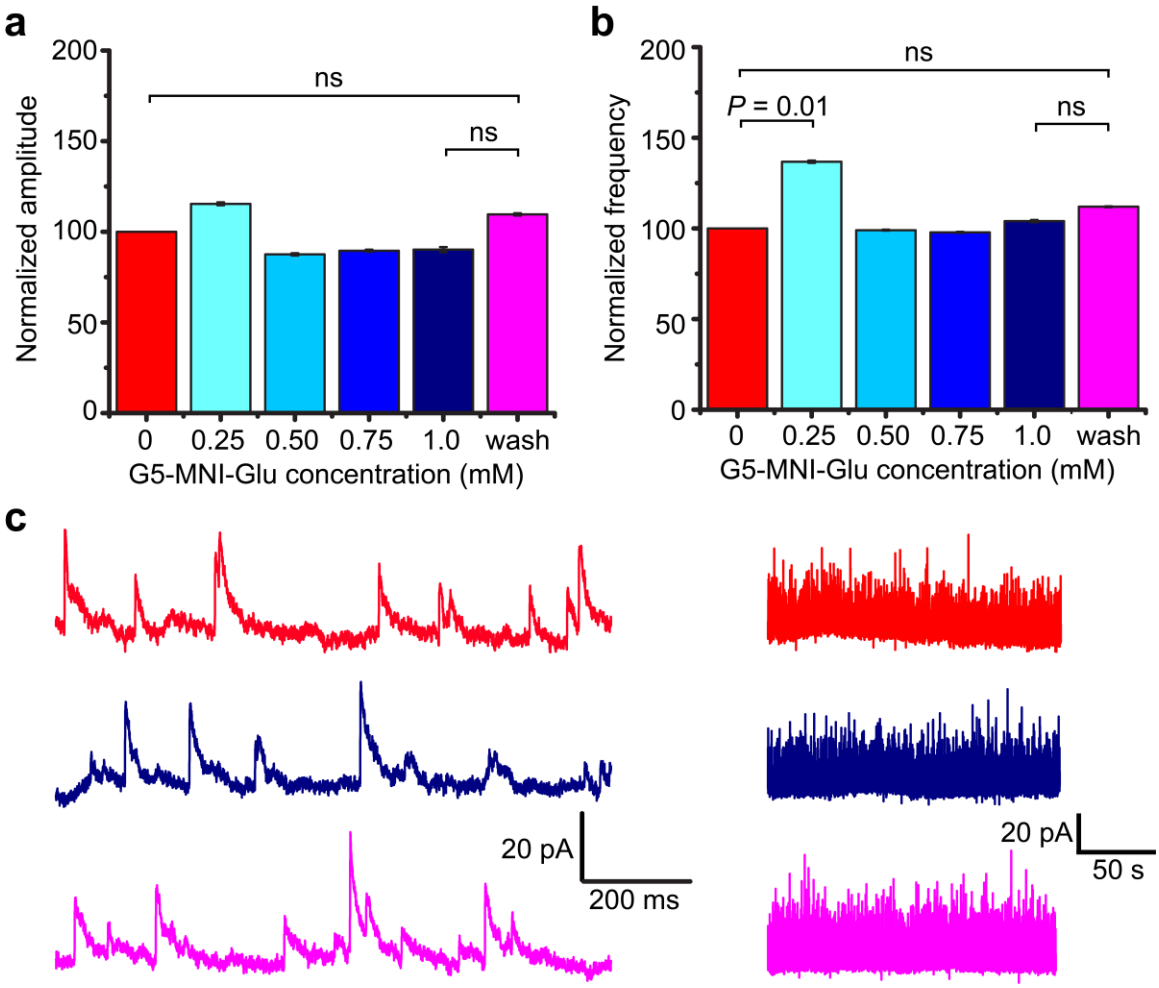


Figure 3. G5-MNI-Glu is non-antagonistic against GABA-A receptors.

Miniature inhibitory postsynaptic currents (mIPSCs) from CA1 neurons were recorded in presence of 0, 0.25, 0.50, 0.75 and 1.0 mM G5-MNI-Glu. The probe was applied in a circulating solution (10 mL) bathing each brain slice. Cells were monitored for 20 min, after an initial period of 4 min. The final wash with normal buffer lasted 20 min. (a,b) Normalized amplitude and frequency for each concentration (n = 3 cells). (c) Representative mIPSCs recordings with [G5-MNI-Glu} = 0 (red) and 1 mM (dark blue), followed by washout (i.e. 0 mM, pink).

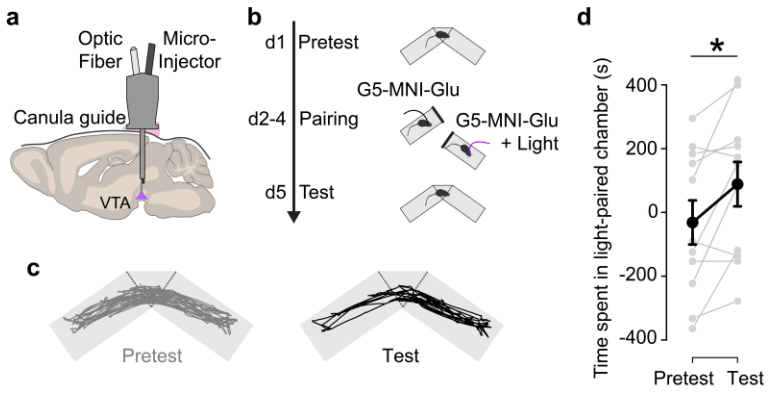


Figure 4. Optofluidic uncaging in freely moving mice.

(a) Cartoon of optofluidic device in the mouse brain. (b) Outline of the conditioned-place preference protocol: "pretest" on day 1 (d1) when implanted mice explore both chambers freely for 900 s; "pairing" (d2-4) when G5-MNI-Glu was infused with or without light; "test" (d5) when mice were allowed to explore the re-connected chambers freely for 900 s (no infusion, no light). (c) Representative trajectories of a mouse before conditioning (pretest) and after conditioning (test). (d) Average (black) of the time spent in the reinforced chamber (G5-MNI-Glu + light) before (pretest) and after (test) after pairing. Individual mice (11) shown in grey.

Acknowledgements.
This work was supported by grants from the NIH to GCRED, from the Fondation pour la Recherche Médicale FRM (Equipe FRM DEQ2013326488) to PF, and from the Brain and Behavior Research Foundation (NARSAD Young Investigator Grant) and the Fondation de France (Fondation Médisite) to AM. RDC was recipients of a fourth year PhD fellowship from FRM (FDT20170437427).

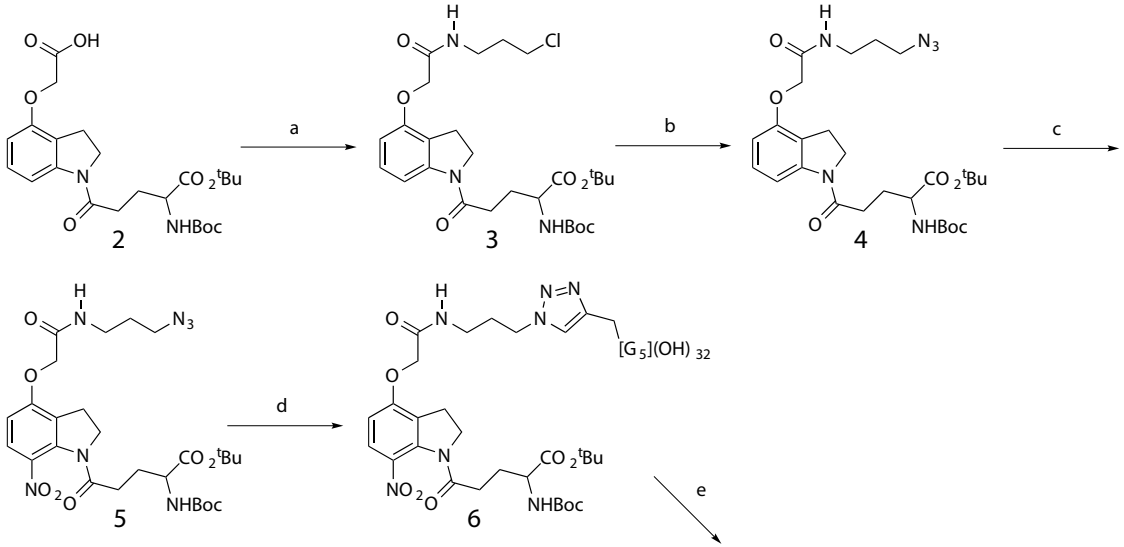
Conflicts of interest.
None.

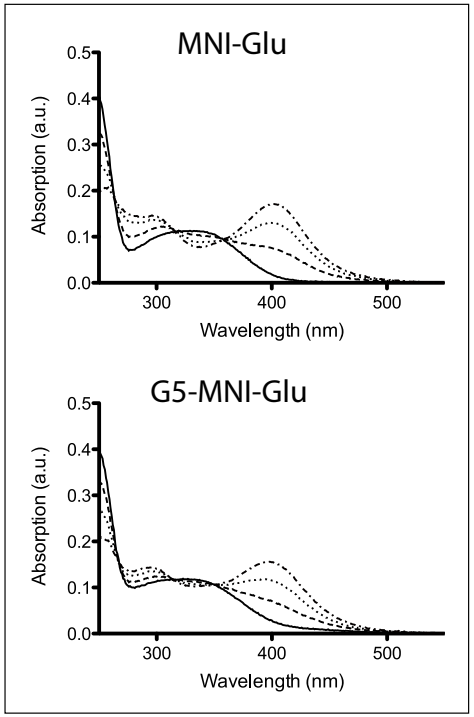
References.

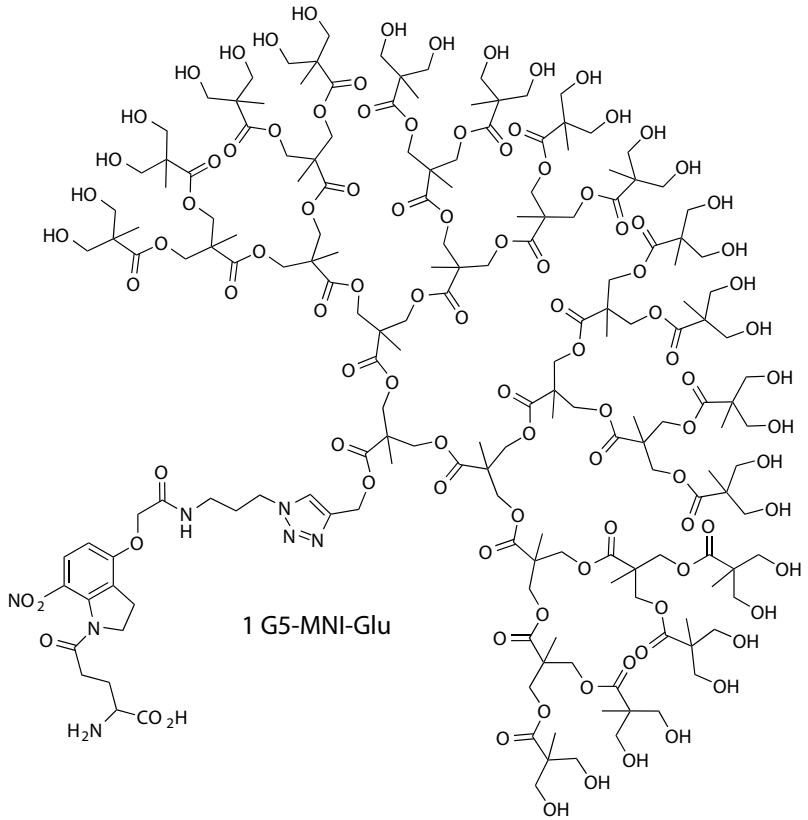
1. G. C. R. Ellis-Davies, Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat Methods* **4**, 619-628 (2007).
2. J. M. Amatrudo, J. P. Olson, H. K. Agarwal, G. C. R. Ellis-Davies, Caged compounds for multichromic optical interrogation of neural systems. *Eur J Neurosci* **41**, 5-16 (2015).
3. J. H. Kaplan, A. P. Somlyo, Flash photolysis of caged compounds: new tools for cellular physiology. *Trends Neurosci* **12**, 54-59 (1989).
4. A. M. Gurney, H. A. Lester, Light-flash physiology with synthetic photosensitive compounds. *Physiol Rev* **67**, 583-617 (1987).
5. S. R. Adams, R. Y. Tsien, Controlling cell chemistry with caged compounds. *Annu Rev Physiol* **55**, 755-784 (1993).
6. G. Mayer, A. Heckel, Biologically active molecules with a "light switch". *Angew Chem Int Ed* **45**, 4900-4921 (2006).
7. H.-M. Lee, D. R. Larson, D. S. Lawrence, Illuminating the chemistry of life: design, synthesis, and applications of "caged" and related photoresponsive compounds. *ACS Chem Biol* **4**, 409-427 (2009).
8. C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer, A. Heckel, Light-controlled tools. *Angew Chem Int Ed* **51**, 8446-8476 (2012).
9. J. D. Clyne, G. Miesenböck, Sex-specific control and tuning of the pattern generator for courtship song in Drosophila. *Cell* **133**, 354-363 (2008).
10. C. Wyart *et al.*, Optogenetic dissection of a behavioural module in the vertebrate spinal cord. *Nature* **461**, 407-410 (2009).
11. D. W. Godwin, D. Che, D. M. O'Malley, Q. Zhou, Photostimulation with caged neurotransmitters using fiber optic lightguides. *Journal of Neuroscience Methods* **73**, 91-106 (1997).
12. S. E. Crowe, S. Kantevari, G. C. R. Ellis-Davies, Photochemically initiated intracellular astrocytic calcium waves in living mice using two-photon uncaging of IP(3). *ACS Chem Neurosci* **1**, 575-585 (2010).
13. J. Noguchi *et al.*, In vivo two-photon uncaging of glutamate revealing the structure-function relationships of dendritic spines in the neocortex of adult mice. *J Physiol* **589**, 2447-2457 (2011).
14. R. Durand-de Cuttoli *et al.*, Manipulating midbrain dopamine neurons and reward-related behaviors with light-controllable nicotinic acetylcholine receptors. *Elife* **7** (2018).
15. J. G. McCall *et al.*, Preparation and implementation of optofluidic neural probes for in vivo wireless pharmacology and optogenetics. *Nat Protoc* **12**, 219-237 (2017).
16. S. Park *et al.*, One-step optogenetics with multifunctional flexible polymer fibers. *Nat Neurosci* **20**, 612-619 (2017).
17. E. M. Callaway, L. C. Katz, Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc Natl Acad Sci U S A* **90**, 7661-7665 (1993).
18. M. Wilcox *et al.*, Synthesis of photolabile precursors of amino acid neurotransmitters. *J. Org. Chem.* **55**, 1585-1589 (1990).

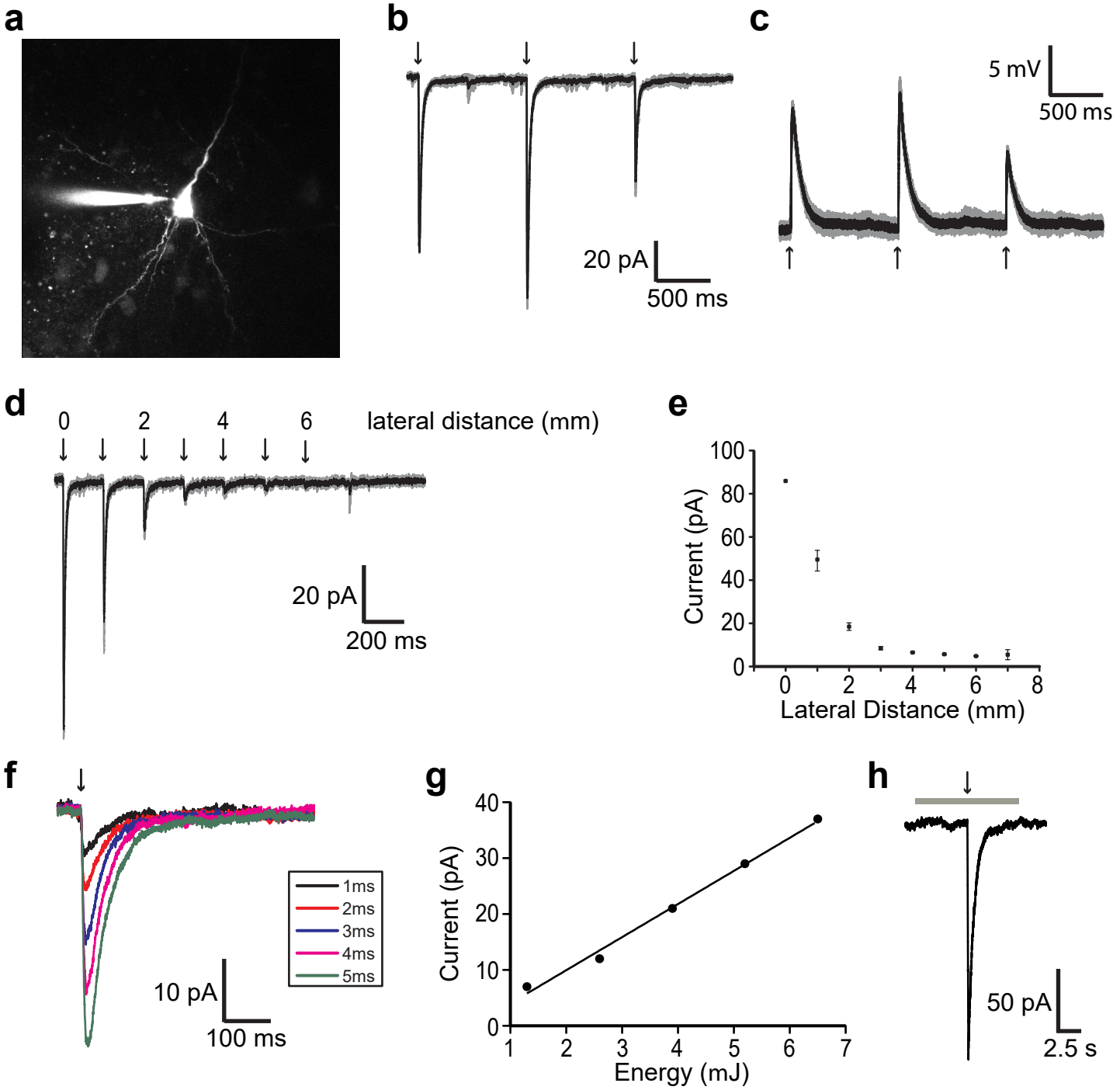
19. R. Wieboldt *et al.*, Photolabile precursors of glutamate: synthesis, photochemical properties, and activation of glutamate receptors on a microsecond time scale. *Proc Natl Acad Sci U S A* **91**, 8752-8756 (1994).
20. M. Canepari, L. Nelson, G. Papageorgiou, J. Corrie, D. Ogden, Photochemical and pharmacological evaluation of 7-nitroindolinyl-and 4-methoxy-7-nitroindolinyl-amino acids as novel, fast caged neurotransmitters. *J Neurosci Meth* **112**, 29-42 (2001).
21. E. Fino *et al.*, RuBi-Glutamate: Two-Photon and Visible-Light Photoactivation of Neurons and Dendritic spines. *Front. Neural Circuits* **3**, 2 (2009).
22. M. Matsuzaki *et al.*, Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* **4**, 1086-1092 (2001).
23. G. C. R. Ellis-Davies, Two-Photon Uncaging of Glutamate. *Front Synaptic Neurosci* **10**, 48 (2019).
24. G. Papageorgiou, J. E. T. Corrie, Synthesis of an anionically substituted nitroindoline-caged GABA reagent that has reduced affinity for GABA receptors. *Tetrahedron* **63**, 9668-9676 (2007).
25. F. F. Trigo, G. Papageorgiou, J. E. T. Corrie, D. Ogden, Laser photolysis of DPNI-GABA, a tool for investigating the properties and distribution of GABA receptors and for silencing neurons in situ. *J. Neurosci. Meth.* **181**, 159-169 (2009).
26. M. Matsuzaki, T. Hayama, H. Kasai, G. C. R. Ellis-Davies, Two-photon uncaging of gamma-aminobutyric acid in intact brain tissue. *Nat Chem Biol* **6**, 255-257 (2010).
27. J. P. Olson *et al.*, Optically selective two-photon uncaging of glutamate at 900 nm. *J Am Chem Soc* **135**, 5954-5957 (2013).
28. J. M. Amatrudo *et al.*, Wavelength-selective one- and two-photon uncaging of GABA. *ACS Chem Neurosci* **5**, 64-70 (2014).
29. M. T. Richers, J. M. Amatrudo, J. P. Olson, G. C. R. Ellis-Davies, Cloaked caged compounds: chemical probes for two-photon optoneurobiology. *Angew Chem Int Ed* **56**, 193-197 (2017).
30. G. C. R. Ellis-Davies, A practical guide to the synthesis of dinitroindolinyl-caged neurotransmitters. *Nat Protoc* **6**, 314-326 (2011).
31. G. Papageorgiou, D. C. Ogden, A. Barth, J. E. T. Corrie, Photorelease of carboxylic acids from 1-acyl-7-nitroindolines in aqueous solution: Rapid and efficient photorelease of L-glutamate. *J Am Chem Soc* **121**, 6503-6504 (1999).
32. G. Papageorgiou, J. Corrie, Effects of aromatic substituents on the photocleavage of 1-acyl-7-nitroindolines. *Tetrahedron* **56**, 8197-8205 (2000).
33. O. D. Fedoryak, J. Y. Sul, P. G. Haydon, G. C. R. Ellis-Davies, Synthesis of a caged glutamate for efficient one- and two-photon photorelease on living cells. *Chem Commun*, 3664-3666 (2005).
34. G. C. R. Ellis-Davies, M. Matsuzaki, M. Paukert, H. Kasai, D. E. Bergles, 4-Carboxymethoxy-5,7-dinitroindolinyl-Glu: an improved caged glutamate for expeditious ultraviolet and two-photon photolysis in brain slices. *J Neurosci* **27**, 6601-6604 (2007).
35. J. Corrie, J. H. Kaplan, B. Forbush, D. C. Ogden, D. R. Trentham, Photolysis quantum yield measurements in the near-UV; a critical analysis of 1-(2-nitrophenyl)ethyl photochemistry. *Photochem Photobiol Sci* **15**, 604-608 (2016).

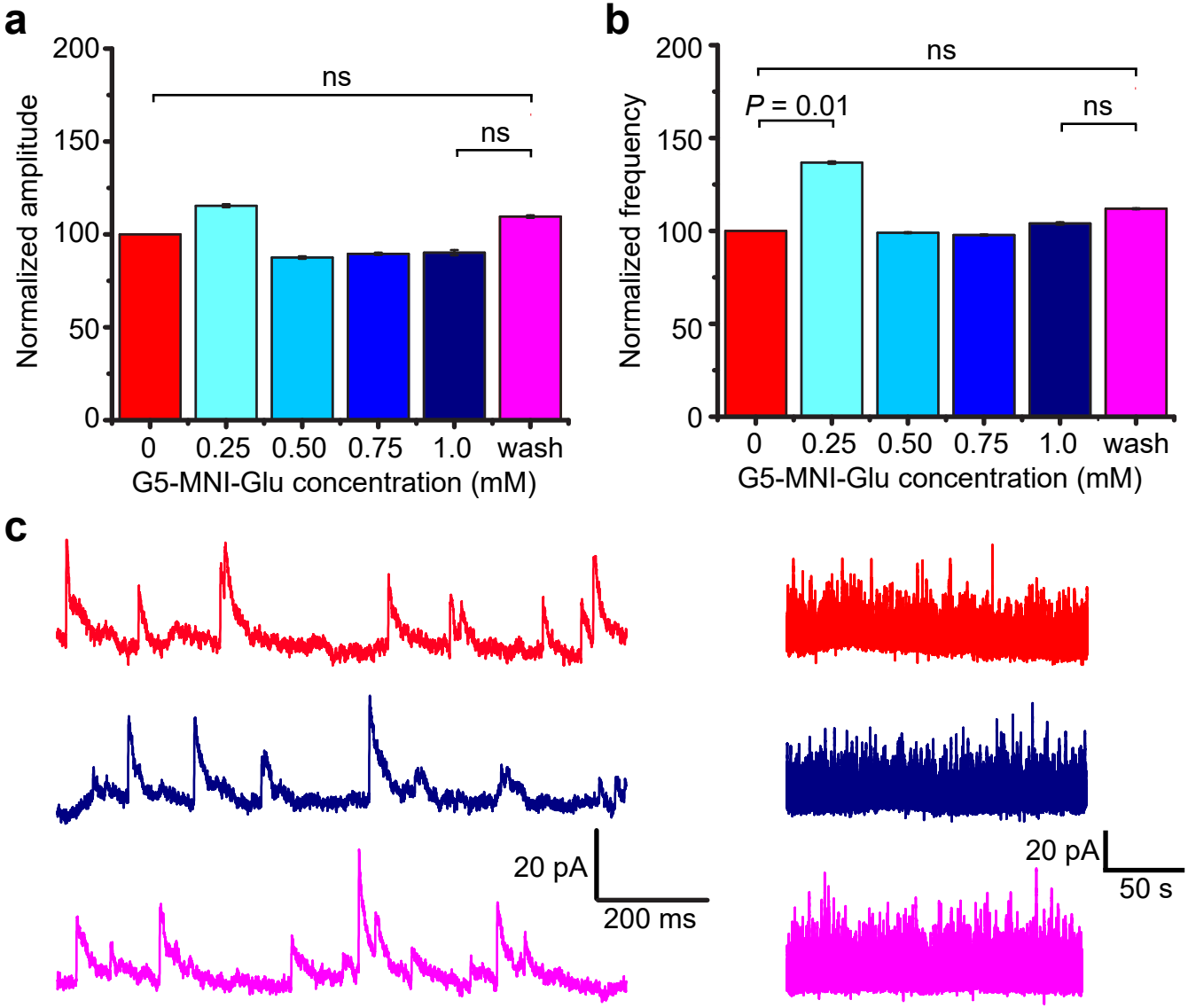
36. S. Passlick, G. C. R. Ellis-Davies, Comparative one- and two-photon uncaging of MNI-glutamate and MNI-kainate on hippocampal CA1 neurons. *J Neurosci Meth* **293**, 321-328 (2017).
37. S. Passlick, P. F. Kramer, M. T. Richers, J. T. Williams, G. C. R. Ellis-Davies, Two-color, one-photon uncaging of glutamate and GABA. *PLoS One* **12**, e0187732 (2017).
38. L. S. Zweifel *et al.*, Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc Natl Acad Sci U S A* **106**, 7281-7288 (2009).
39. H. C. Tsai *et al.*, Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* **324**, 1080-1084 (2009).
40. S. Kantevari *et al.*, Development of anionically decorated caged neurotransmitters: in vitro comparison of 7-nitroindolinyl- and 2-(p-phenyl-o-nitrophenyl)-propyl-based photochemical probes. *ChemBioChem* **17**, 953-961 (2016).
41. S. H. Medina, M. E. El-Sayed, Dendrimers as carriers for delivery of chemotherapeutic agents. *Chem Rev* **109**, 3141-3157 (2009).
42. D. Astruc, E. Boisselier, C. Ornelas, Dendrimers designed for functions: from physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine. *Chem Rev* **110**, 1857-1959 (2010).
43. W. A. Velema, W. Szymanski, B. L. Feringa, Photopharmacology: beyond proof of principle. *J Am Chem Soc* **136**, 2178-2191 (2012).
44. K. Hull, J. Morstein, D. Trauner, In Vivo Photopharmacology. *Chem Rev* **118**, 10710-10747 (2018).
45. J. Broichhagen, J. A. Frank, D. Trauner, A roadmap to success in photopharmacology. *Acc Chem Res* **48**, 1947-1960 (2015).
46. E. Bamberg, W. Gartner, D. Trauner, Introduction: Optogenetics and Photopharmacology. *Chem Rev* **118**, 10627-10628 (2018).
47. M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, Emerging Targets in Photopharmacology. *Angew Chem Int Ed Engl* **55**, 10978-10999 (2016).
48. P. Paoletti, G. C. R. Ellis-Davies, A. Mourot, Optical control of neuronal ion channels and receptors. *Nat Rev Neurosci* **20**, 514-532 (2019).
49. J. T. Ting, T. L. Daigle, Q. Chen, G. Feng, Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. *Methods Mol Biol* **1183**, 221-242 (2014).

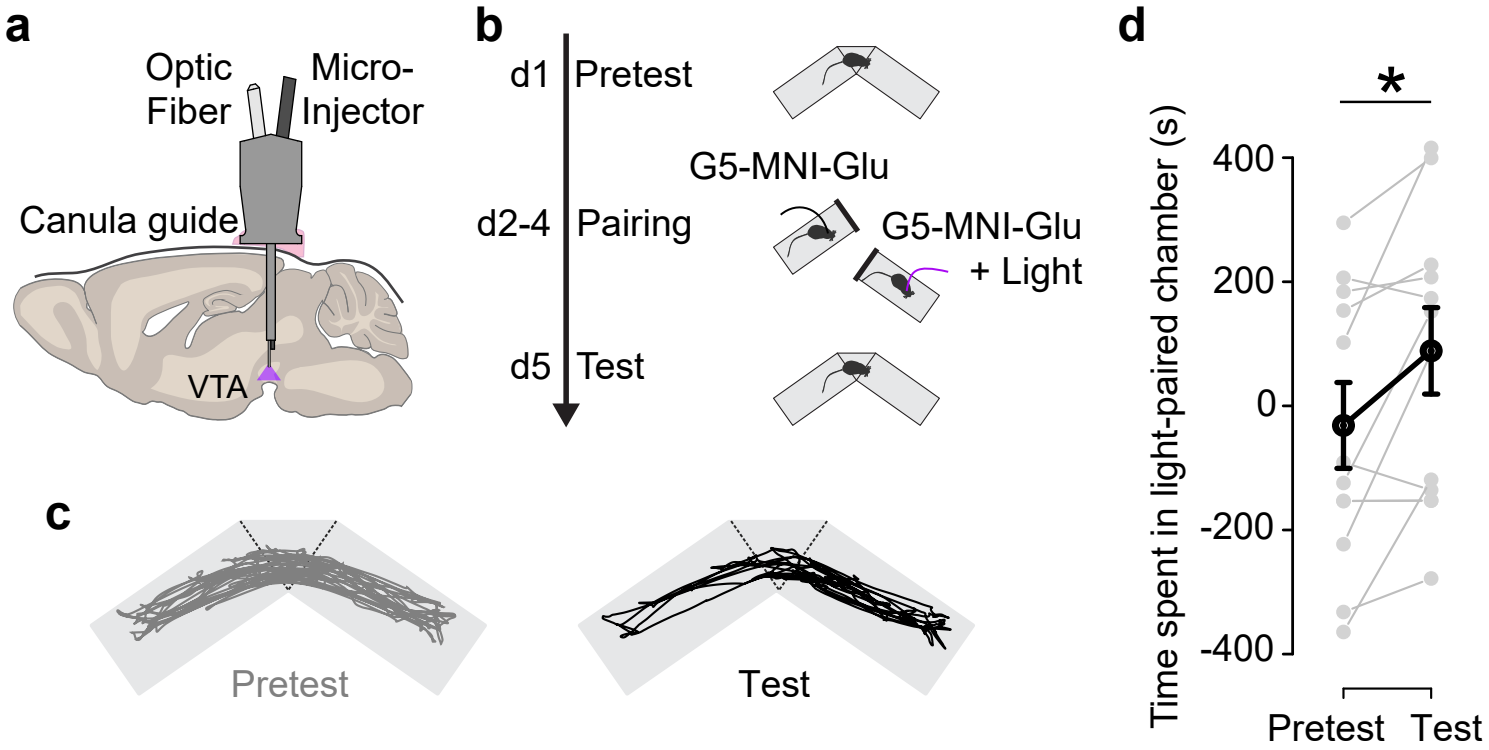

a
b
c
d
e
2
3
4
5
6
NHBoc
$CO_2{}^tBu$
$[G_5](OH)_{32}$


MNI-Glu
G5-MNI-Glu
Absorption (a.u.)
Wavelength (nm)
0.0
0.1
0.2
0.3
0.4
0.5
300
400
500


1 G5-MNI-Glu


a
b
20 pA
500 ms
c
5 mV
500 ms
d
0
2
4
6
lateral distance (mm)
20 pA
200 ms
e
Current (pA)
Lateral Distance (mm)
f
1ms
2ms
3ms
4ms
5ms
10 pA
100 ms
g
Current (pA)
Energy (mJ)
h
50 pA
2.5 s


a
200
150
100
50
0
Normalized amplitude
ns
ns
0
0.25
0.50
0.75
1.0
wash
G5-MNI-Glu concentration (mM)
b
200
150
100
50
0
Normalized frequency
ns
P = 0.01
ns
0
0.25
0.50
0.75
1.0
wash
G5-MNI-Glu concentration (mM)
c
20 pA
200 ms
20 pA
50 s


a
Optic Fiber
Micro-Injector
Canula guide
VTA
b
d1
Pretest
G5-MNI-Glu
d2-4
Pairing
G5-MNI-Glu
+ Light
d5
Test
c
Pretest
Test
d
*
Time spent in light-paired chamber (s)
400
200
0
-200
-400
Pretest
Test