# DEVELOPMENT OF A NEW PHOTOCHROMIC ION CHANNEL BLOCKER VIA AZOLOGIZATION OF FOMOCAINE

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**ABSTRACT:**

Photochromic blockers of voltage gated ion channels are powerful tools for the control of neuronal systems with high spatial and temporal precision. We now introduce fotocaine, a new type of photochromic channel blocker based on the long-lasting anestheticfomocaine. Fotocaine is readily taken up by neurons in brain slices and enables the optical control of action potential firing by switching between 350 and 450 nm light. It also provides an instructive example for "azologization", that is, the systematic conversion of an established drug into a photo switchable one.

**KEYWORDS:** Photo pharmacology, local anesthetics, fomocaine, action potential firing control, azologization, azobenzene photo switch.

**INTRODUCTION:**

Optical methods for controlling neuronal function have revolutionized neuroscience in recent years.1−3 For instance, photoswitchable versions of local anesthetics have proven to be powerful tools for addressing native voltage-gated ion channels4,5 and have been used in pain research and vision restoration.6−8 Two of these compounds, namely, the bis-quaternary ammonium ion QAQ6 and the quaternary ammonium ion DENAQ8 are in essence photoswitchableazobenzene derivatives of QX-314, which in itself is a permanently charged version of lidocaine (Figure 1).9 The positive charge of these photoswitchable ion channel blockers limits their ability to cross biological barriers. As a consequence, QAQ needs to be loaded through the patch pipet or imported via accessory ion channels, such as P2X7 or TRPV1. This can be an advantage when the investigation of a single cell is desired, but may complicate the application of QAQ in tissues where these pathways are not available.6 DENAQ only bears one permanent charge and does not require accessory ion channels to exert its biological effects, for example, on HCN channels,8 but is somewhat limited in its ability to diffuse through tissues. In our ongoing efforts to develop new and improved photo switchable ion channel blockers, we therefore decided to investigate alternative pharmacophores, which may facilitate biodistribution and have advantageous pharmacokinetic properties Local anesthetics have a long medical history and are mechanistically reasonably well understood.10−13 They usually function as use-dependent open channel blockers, in particular of voltage gated-sodium channels (NaV), but can also have effects on other molecular targets. The alkaloid cocaine, for instance, has been used as a topical anesthetic in ophthalmology since the turn of the last century, despite its well-known effects on the central nervous system.14 Novocaine is a representative of

the so-called ester local anesthetics and was initially developed as a simplified analogue of cocaine. In the late 1960s, the morpholinefomocaine was introduced, which bears little structural resemblance to cocaine with the exception of a tertiary amine functionality.14,15 Like lidocaine and novocaine, it is not permanently charged but it is much more lipophilic and shows reduced systemic toxicity. As such, it has been used for decades as a long-lasting topical anesthetic.14 We now describe a photoswitchable version of fomocaine, termed fotocaine, which functions as a photochromic ion channel blocker and can be used to control neuronal activity with light.

In addition to its interesting pharmacological properties, fomocaine evoked our interest due to its molecular structure. It features a benzyl-phenyl ether moiety and thus abides to our philosophy of "azologization", that is, the rational introduction of azobenzenes into drugs (Figure 2). Obvious targets for azologization are compounds that incorporate stilbenes, 1,2-diphenyl ethanes, 1,2-diphenyl hydrazines, N-benzyl anilines, benzyl-phenyl ethers, benzyl-phenyl thioethers, diaryl esters, diaryl amides, and heterocyclic derivatives thereof (Figure 2a). A survey of databases shows that many established drugs feature these moieties. Replacing them with azobenzenes yields photoswitchable analogues that resemble their parent compounds in size and shape ("azosters"), but ideally change their efficacy upon irradiation. Application of this logic to fomocaine yields fotocaine, wherein a CH2−O moiety has been replaced by a diazene unit (N□N) (Figure 2b). We hypothesized that this substitution would provide a photoswitchable ion channel blocker with similar pharmacodynamics and pharmacokinetics. The three-step synthesis

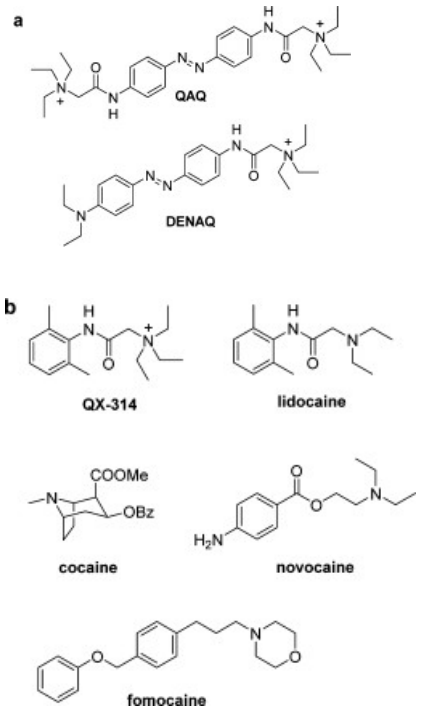


Figure 1. (a) Photoswitchableazobenzene derivatives QAQ and DENAQ used as photoswitchable blockers of voltage gated ion channels. (b) Structures of local anesthetics. QX-314 is a permanently charged derivative of the local anestheticlidocaine. Cocaine is a tropane alkaloid, and novocaine is an ester local anesthetic. All of these compounds feature a tertiary amine pharmacophore. Fomocaine is an ether local anesthetic with a morpholino group

of fotocaine from commercially available starting materials is described in the Supporting Information. To test fotocaine'sphotoswitching properties, we first utilized UV/vis spectroscopy (Figure 3). A 50 μM solution of fotocaine in DMSO was placed in a quartz cuvette with 1 cm diameter and illuminated from above using a monochromator. The lightinduced isomerization of fotocaine and the corresponding absorption spectra of cis- and trans-isomers are depicted in Figure 3a. As a classical azobenzene,

isomerization could be followed by monitoring the π to π* transition at 330 nm over time. Toggling between 350 and 450 nm light switched the molecule into its cis- and trans-state, respectively (Figure 3c, i). As it is known for regular azobenzenes, photostationarycis/trans ratios of up to 90:10 can be achieved by irradiation with ultraviolet light.3 Wavelengths between 400 and 350 nm could be used to install mixtures with different cis/trans ratios (Figure 3c, ii). As expected from a "classical" azobenzene, the thermodynamically less stable cis-state remained stable in the dark (Figure 3c, iii).16,17 Thus, fotocaine provides the desired reversible light-mediated cis/trans-isomerization. As an added advantage, it shows bistability and stays in its cis-state for several minutes even without continuous UV-illumination.

Next, we investigated the ability of fotocaine to optically control neuronal function. To this end, we resorted to patch clamp electrophysiology using dissociated mouse hippocampal neurons (Figure 4). Fotocaine was applied at 50 μM concentration in the external bath solution. At a starting potential of −80 mV, action potential (AP) firing of neurons was induced by injecting a 50 pA current. When the illumination wavelength was set to 450 nm, AP firing was inhibited. However, when switching to 350 nm, AP firing triggered by the same current took place reliably. This process could be repeated with a variety of different illumination protocols (Figure S1). The same effects were observed at higher concentration (100 μMfotocaine, Figure S1b). The single action potential at the beginning of each current injection under 450 nm indicates that trans-fotocaine acts as an open channel blocker as is the case for its permanently charged relatives.9,18 To test the action of fotocaine in a functional neuronal circuit and assess its distribution in tissues, we performed further patch clamp experiments using acute hippocampal mouse brain slices.

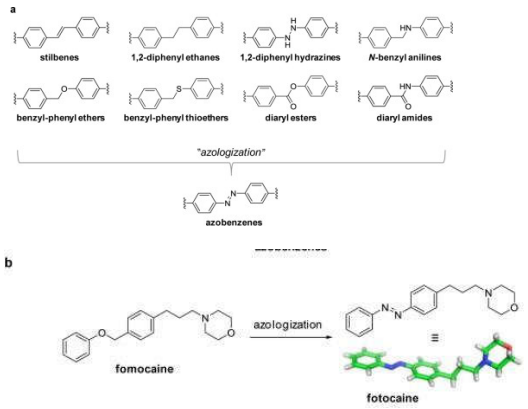


Figure 2. Logic of azologization. (a) Prime isosters of azobenzenes, that is, azosters, are stilbenes, 1,2-diphenyl ethers, 1,2.diphenyl hydrazines, N-benzyl anilines, benzyl-phenyl ethers, benzyl-phenyl thioethers, diaryl esters, and diaryl amides. (b) Application of the concept of azologization to fomocaine. Replacement of the benzyl-phenyl ether bridge by a diazene yields the azobenzene derivative fotocaine. The X-ray structure of fotocaine is deposited at the Cambridge crystallographic data center, ID: 991565.

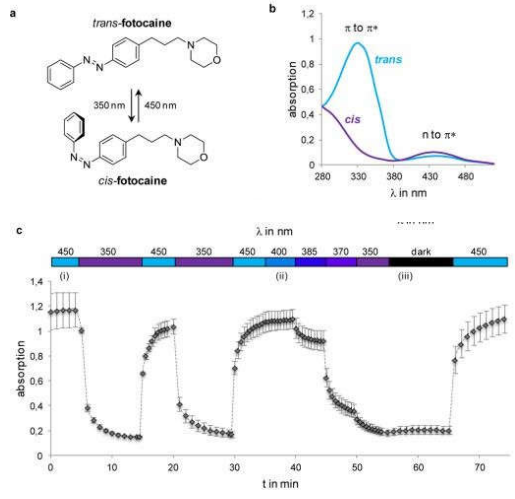


Figure 3. Photoswitching of fotocaine followed by UV/vis spectroscopy. (a) UV-light (e.g., 350 nm) isomerizes the azobenzene functional group in fotocaine to itscis-isomer, which is the

thermodynamically less stable state. Blue light (e.g., 450 nm) triggers isomerization to trans. (b) Absorption spectra of trans-fotocaine (blue line) and cis-fotocaine (purple line) are distinct. The π to π* band decreases starkly upon isomerization to cis, while the n to π* band slightly increases. (c) In-time photoswitching by following the fotocaine absorption at 330 nm. Fotocaine can be reversibly isomerized by switching between, for example, 450 and 350 nm (i). Wavelengths between 400 and 350 nm lead to graded effects (ii). Once switched to cis, fotocaine stays in its excited state without further illumination (iii). No decay was detected for the investigated time of 10 min (n = 3, error bar indicates standard deviation).

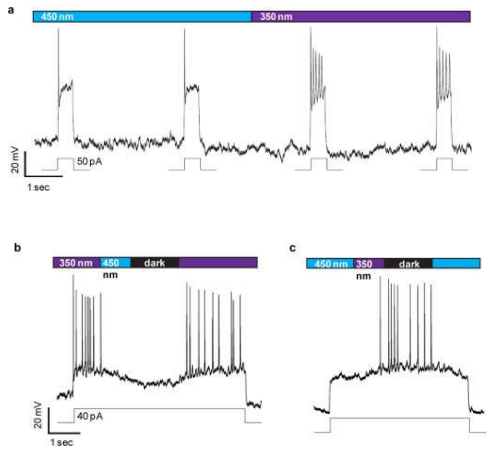


Figure 4. Photocontrol of action potential (AP) firing mediated by fotocaine investigated in whole cell patch clamp experiments (representative traces). (a) Dissociated mouse hippocampal neurons, current clamp mode, 50 μMfotocaine. AP-firing was triggered by injecting 50 pA for 300 ms (cells were held at −80 mV). Under 450 nm (trans-fotocaine), AP-firing was suppressed, while 350 nm illumination (cis-fotocaine) allowed AP-firing. The initial AP under 450 nm is indicative of the action of an open channel blocker. (b and c) Acute mouse brain slice, hippocampal CA1 neurons, current clamp mode, after 10 min wash-out of fotocaine. Currents of 40 pA were injected, and illumination wavelengths were changed simultaneously. Effects of cis- and trans-fotocaine were identical to those in (a). In addition, when 350 or 450 nm was turned off after short application, the thereby installed effect maintained.

First, the tissue preparation was treated with 100 μMfotocaine for 5 min to allow the cells to take up the photochromic drug. Then, buffered ringer solution was perfused for 10 min to remove the photoswitchable blocker from the extracellular solution. As expected for a long-lasting open channel blocker, photocontrol of AP firing was possible without continuous supply of extracellular fotocaine (Figure 4b). AP firing was triggered by injecting a 40 pA current for several seconds, and illumination wavelengths were switched during this activation period. In line with our observations using dissociated neurons, AP firing was inhibited by 450 nm illumination and enabled by 350 nm. Furthermore, the bistability of fotocaine, which was established with UV/vis spectroscopy (Figure 3c), also applied to this physiological experiment. Neuronal silencing triggered by 450 nm light was sustained in the dark but could be lifted with 350 nm light (Figure 4b). Conversely, AP-firing activated with 350 nm light continued after the light was tuned off but could be abrogated by switching to 450 nm (Figure 4c).

In summary, we have applied the logic of azologization to the local anestheticfomocaine, thus establishing a novel photochromic ion channel blocker, fotocaine. We demonstrated that fotocaine gets readily taken up by neurons in brain slices, can be used to control their action potential firing with light, and has long-lasting effects. Its relatively simple

structure could facilitate the design and synthesis of improved versions with desirable properties, such as red-shifted action spectra. These investigations and the application of fotocaine in neurophysiology, in particular as an analgesic for the photopharmacological control of pain, will be reported in due course.

## METHODS

UV/Vis Spectroscopy. UV/vis spectroscopy was performed using a VARIAN Cary 50 Scan UV/vis spectrometer. PCL solution was placed in a standard quartz cuvette (d = 1 cm) illuminated by a light fiber cable from above.

**Cell and Tissue Preparation.** Dissociated mouse hippocampal neurons were prepared and cultured using an astrocyte feeder layer as reported elsewhere.19 For acute mouse hippocampal brain slices, BL6 wild type mice (postnatal days 9−13) of either sex were quickly decapitated, the brain was removed, and 250 μm horizontal slices were prepared using a vibrating microtome (7000smz-2, Campden Instruments). Slices were incubated for 30 min at 34 °C in carbogenated (5% CO2, 95% O2) sucrose medium (mM: 87 NaCl, 2.5 KCl, 7 MgCl2, 0.5 CaCl2, 25 Gluc, 1.25 NaH2PO4, 25 NaHCO3, 75 sucrose, (319 mOsm)). Slices were perfused with 100 μMfotocaine in bath solution for 5 min, followed by 10 min perfusion with bath solution. Whole cell patch clamp recordings where performed on CA1 hippocampal neurons.

**Electrophysiology.** Whole cell patch clamp recordings where performed using a standard electrophysiology setup equipped with a HEKA Patch Clamp EPC10 USB amplifier and patch master software. Micropipets were generated from "Science Products GB200-F-8P with filament" pipets using a vertical puller (PC-10, Narishige). Resistance varied between 5 and 7 MΩ. Bath solution for dissociated hippocampal neurons contained the following in mM: 140 NaCl, 3 KCl, 2 CaCl2, 1 MgCl2, D-Gluc 10, 20 HEPES (NaOH to pH 7.4). Pipet solution for dissociated hippocampal neurons contained the following in mM: 107 KCl, 1.2 MgCl2, 1 CaCl2, 10 EGTA, 5 HEPES, 2 MgATP, 0.3 Na2GTP (KOH to pH 7.2). Bath solution for acute brain slice contained the following in mM: 125 NaCl, 2.5 KCl, 1 MgCl2, 2 CaCl2, 10 Glu, 1.25 NaH2PO4, 26 NaHCO3, (290−295 mOsm). Pipet solution for acute brain slice contained the following in mM: 140 K-gluconate, 4 NaCl, 12 KCl, 10 HEPES, 4 MgATP, 0.4 Na2ATP (KOH to pH 7.3). Action potentials (APs) were induced with 50 pA current injection. Fotocaine was dissolved in bath solution from a 1000× DMSO stock for either tissue preparation.

**Illumination.** Irradiation during electrophysiology and UV/vis experiments was performed using a TILL Photonics Polychrome 5000 monochromator operated by the PolyCon software and by using the patch clamp amplifier, respectively.

## ASSOCIATED CONTENT

### *S Supporting Information

Representative traces of AP-firing using different concentrations of fotocaine and illumination timing (Figure S1). Synthesis and characterization of organic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

## ACKNOWLEDGMENTS

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