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A new gustometer: Template for the construction of a portable and modular stimulator for taste and lingual touch

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Abstract

Taste research has been hampered by technical difficulties, mostly because liquid taste stimuli are difficult to control in terms of timing and application area. Exact stimulus control requires a gustometer; yet, existing devices are either not well-documented or rather inflexible. We designed a gustometer based on a computer-controlled, modular pump system, which can be extended through additional hardware modules, e.g. for heating of the stimuli or sending and receiving triggers. All components are available for purchase “off-the-shelf”. The pumps deliver liquids through plastic tubing and can be connected to commercially available or custom-made mouthpieces. We determined the temporal precision of the device. Onset delay showed minuscule variation within pumps (SD < 3 ms) and small differences between pumps (< 4.5 ms). Rise time was less than 2 ms (SD < 2 ms). Dosage volume bias was only 2%. To test whether the hemitongues could be stimulated independently, we conducted a behavioral experiment. 18 participants received tasteless stimuli to the left, right, or both sides of the tongue. The side of stimulation was correctly identified in 91% of the trials, indicating that the setup is suitable for lateralized stimulation. EEG responses to water and salty stimuli were recorded from two participants; the stimulation successfully evoked event-related responses, demonstrating the suitability of the device for use in electrophysiological investigations. We provide a Python-based open-source software package and a web interface to easily operate the system. We thereby hope

to facilitate access to state-of-the-art taste research methods and to increase reproducibility across laboratories.

Keywords: gustometer, taste stimulator, taste, stimulator, gustation, flavor, touch

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Introduction

Despite the growing interest in gustatory processing, owing to its significance in food preference and food intake, the sense of taste remains the least understood sensory system. A major contributing factor is the difficulty to present liquid taste (or flavor) stimuli in a controlled manner. However, precisely controlled stimulus delivery with steep onset flanks is necessary for behavioral response time measurements and electrophysiological investigations in order to take full advantage of the high temporal resolution these methods can provide. Furthermore, exact temporal alignment of stimuli across trials is vital to studies of evoked potentials, in which the summed responses of single trials are analyzed, and poor timing inevitably impairs signal-to-noise ratios. Precise stimulus control is also crucial to ensure reproducible stimulation within and between participants and across experimental sessions. The solution is to automate stimulus delivery with the help of a programmable taste stimulator – a *gustometer* – instead of presenting the stimuli manually, e.g. using hand-operated pipettes.

Before gustometers became commercially available, studies on taste perception required development and construction of custom-made devices. For example, the first gustatory event-related potentials to liquid stimuli were obtained almost 50 years ago using a hinged spoon pouring liquids onto the tongue (Funakoshi & Kawamura, 1971). Since then, numerous devices for gustatory stimulation have been designed, be it for behavioral (Ashkenazi, Fritz, Buckley, & Marks, 2004), hemodynamic (Veldhuizen, Bender, Constable, & Small, 2007), or electrophysiological investigations (Kobayakawa et al., 1996; also see Ohla, Busch, & Lundström, 2012 for a review).

Some researchers have presented stimuli via a sponge (Wada, 2005) or used syringe pumps to deliver liquids through a set of tubes directly onto the tongue (Andersen et al., 2018; Franken et al., 2010). This approach concomitantly activates the gustatory and the lingual tactile systems. To isolate the gustatory response, other researchers have embedded taste stimuli within a stream of tasteless stimuli, thereby minimizing or even abolishing lingual somatosensory responses through adaptation and habituation. Kobal (1985), for example, used an olfactometer to deliver gaseous stimuli embedded in a constant flow of tasteless air to the mouth. Similarly, the gustometers used by Plattig, Dazert, & Maeyama (1988) and Gerull, Mrowinski, & Schilling (1984) embedded aqueous stimuli in a continuous flow of water. To ensure that consecutive stimuli do not mix or dilute, Kobayakawa et al. (1996) inserted small air bubbles before and after each stimulus, thereby separating taste

stimuli from the tasteless background solution. However, replication of the previously mentioned setups is often impeded by only partial documentation and lack of commercial availability of the used devices.

More recently, a gustometer that delivers liquids as clearly separated atomized spray pulses became commercially available (GU002, Burghart, Wedel, Germany; see Iannilli, Beger, Fürer, & Hummel, 2015 for a description of this device). With this system, liquid tastants are embedded in a regular series of water or rinse pulses, desensitizing tactile perception over time and thereby producing a purely unimodal taste stimulation without concomitant tactile activation. The pulse sequence can be configured to exclude mixing of stimuli and to provide sufficient temporal precision to elicit gustatory event-related potentials, which have been observed in different laboratories (see e.g. Crouzet, Busch, & Ohla, 2015; Iannilli, Broy, Kunz, & Hummel, 2017; Tzieropoulos, Rytz, Hudry, & le Coutre, 2013). The introduction of the Burghart gustometer, for the first time, offered users access to taste research, without requiring the resources or skills to build their own gustometer. However, its relatively poor dissemination – only a few research groups worldwide use the device – indicates that it comes with certain drawbacks. The acquisition and maintenance costs are not affordable to everybody. The number of pumps is fixed (max. five pumps for tastants two pumps for rinse). Further potential limitations are posed by the relatively inflexible proprietary control software and the requirement by the manufacturer that participants must maintain an upright position during stimulation (but cf. e.g. Iannilli, Singh, Schuster, Gerber, and Hummel, 2012, who used a modified version of the GU002 in an fMRI study).

Direct comparison of results from different research groups is often difficult since so many different stimulation approaches have been employed over the past decades. Moreover, development and construction, but also the maintenance of a taste stimulator often require substantial resources. We therefore developed a comparably cheap gustometer sufficient for a variety of experimental applications. It delivers temporally precise and accurate stimuli with steep onset flanks as commonly required in behavioral and electrophysiological investigations. Furthermore, its portability allows easy transport between different experimental locations, such as a behavioral lab and an fMRI scanning facility. The device is modular and can be assembled with commercially available parts. The number of syringe pumps is variable and can be adjusted to the number of stimuli required in the experiment. Syringes can be refilled automatically in the course of an experimental session. Further, the functionality of the setup can be expanded with a multitude of additional modules, e.g. for heating, digital input/output (I/O) etc. All components are easily replaceable, allowing for optimal hygienic practices. The gustometer can be equipped with custom-made

mouthpieces. Stimuli can be delivered to participants in upright or supine position. Lateralized stimulus delivery (i.e., to the left and right side of the tongue separately) can be achieved with a mouthpiece design presented in this paper. We developed a software package *(pyqmix)* to control the gustometer. Its intuitive programmable Python interface allows for great experimental flexibility and excellent integration with existing neuroscientific software packages such as PsychoPy (Peirce, 2009) and expyriment (Krause & Lindemann, 2014). Additionally, we developed a web application *(pyqmix-web)* that simplifies common tasks like filling and emptying the syringes without requiring any programming knowledge. The source code of both software packages is available free of charge, allowing users to modify and extend the functionality if required.

With this publication we aim to provide a template for researchers desiring to assemble and operate a well-documented, versatile, modular, portable, computer-controlled gustometer for research purposes, which can be built at a low entry price and without the need for advanced technical skills.

Methods

A full list of the required components to build the gustometer, along with a price list, is compiled in Table 1. Note that the prices are based on quotes from December 2017, and that other vendors may offer the same or similar products.

Gustometer construction

The gustometer is modular, allowing the experimenter to adjust it to a number of experimental setups. In the following, we will present a configuration with two different mouthpieces, and we supplement the description with highlights of potential alterations and extensions of the system. Important design criteria were the portability of the device and straightforward assembly. While the gustometer could be easily embedded in a cabinet, we implemented a version that can be easily carried and placed on a desk or a table with wheels, which improves accessibility and simplifies adaptations of the gustometer, for example the insertion or removal of a pump module.

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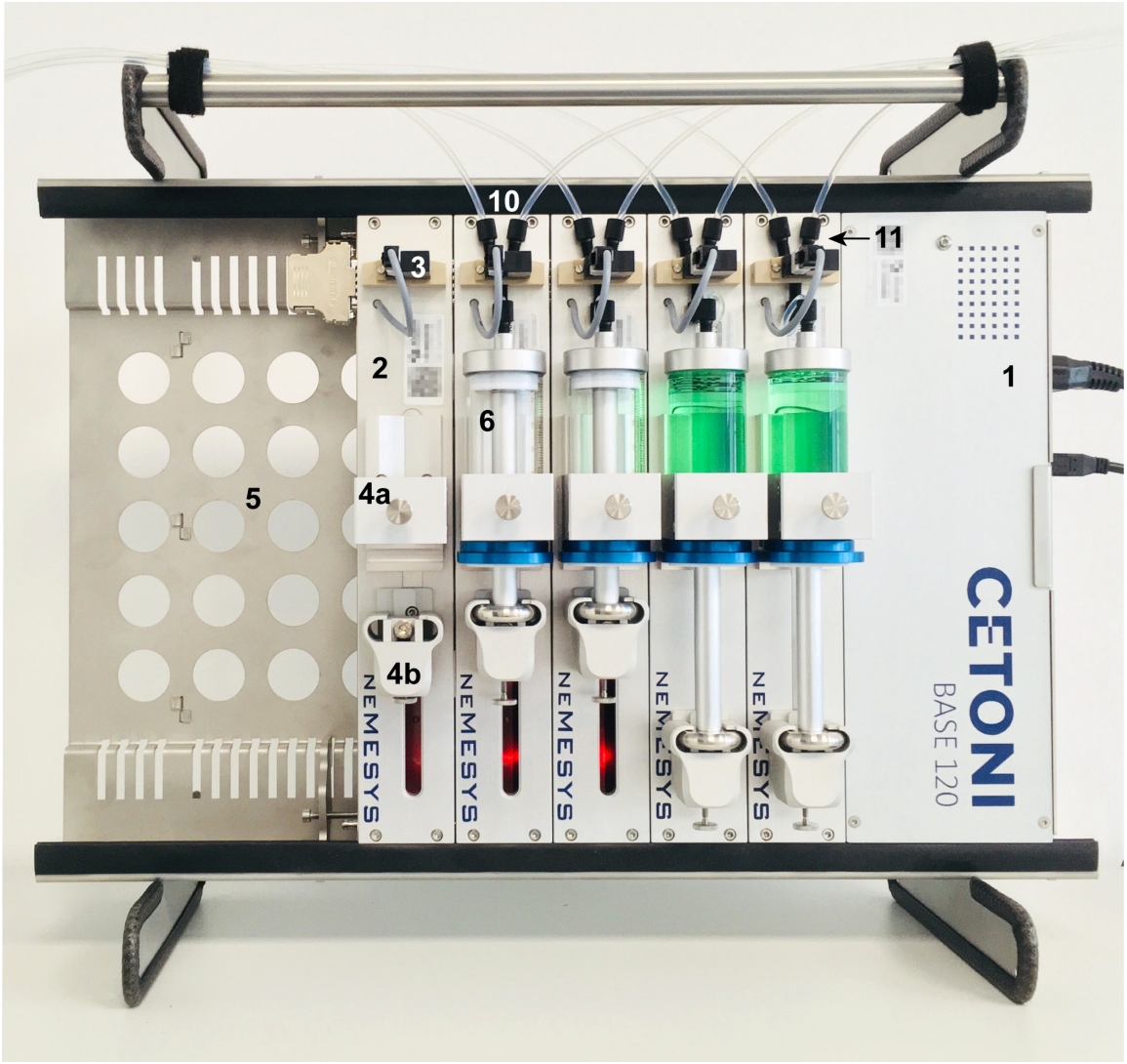

Figure 1. Photograph of the gustometer. The *BASE 120* module (1) and five *neMESYS 290N* low pressure syringe pump modules (2) are mounted in a system clamp (5) in upright position. The installed 50 mL high-precision glass syringes (6) are held in place by syringe holders (4a) and syringe piston holders (4b). The syringes themselves are connected to computer-controlled 3/2-way solenoid valves (3). Leak-proof tubing (10) connections are established through flangeless fittings with accompanying ferrules (11). The power cord and USB cable connect to the right side of the system. The component numbers used in this figure match the numbers in Table 1.

Table 1. Component list.

Identifier	Component	Manufacturer	Distributor	Product no.	Qty.	Price per piece €	Total price €
	Pump setup						
1	BASE 120 module	Cetoni GmbH	Cetoni GmbH	—	1	1,500.00	1,500.00
2	neMESYS 290N low pressure syringe pump	Cetoni GmbH	Cetoni GmbH	—	4	3,300.00	13,200.00
3	3/2-way solenoid low-pressure valve, 0.6 mm diameter*	Cetoni GmbH	Cetoni GmbH	—	4	—	—
4	Syringe holder & syringe piston holder*	Cetoni GmbH	Cetoni GmbH	—	4	—	—
5	System clamp 520 mm	Cetoni GmbH	Cetoni GmbH	—	1	750.00	750.00
6	Glass syringe, 50 mL	SETonic GmbH	Cetoni GmbH	—	4	220.00	880.00
	Software						
	Qmix SDK	Cetoni GmbH	Cetoni GmbH	—	1	800.00	800.00
	Mouthpiece						
7	Inlet check valve	IDEX Health & Science	Techlab	CV-3324	4	123.00	492.00
8	3-way manifold (Y-connector, for lateral tongue stimulation)	IDEX Health & Science	Techlab	P-513	2	39.00	78.00
9	5-way manifold (4 stimulus inputs, for whole-tongue stimulation)	IDEX Health & Science	Techlab	P-155	1	103.00	103.00
	Accessories						
10	Tubing (ETFE, OD**: 1/8", ID**: 1/16")	—****	Techlab	KAP 100.965	30	7.70	231.00
11	Flangeless fittings with accompanying ferrules (packs of 10)	IDEX Health & Science	Techlab	XP-301X	3	28.00	84.00
	Flat-Bottom Plug***	IDEX Health & Science	Techlab	P-309	3	1.60	4.80
	Total						18,118.00

All prices without VAT.

**included with the pump modules*

***OD: outer diameter, ID: inner diameter*

****To clog unused ports in the mouthpiece*

*****Manufacturers may vary.*

Websites

Cetoni GmbH	https://www.cetoni.de
SETonic GmbH	https://setonic.com
IDEX	https://www.idex-hs.com
Techlab	https://techlab.de/

Pump System

The heart of the gustometer is the computer-controlled, CE-certified *neMESYS* low-pressure syringe pump system (Cetoni GmbH, Korbussen, Germany). Its *BASE 120* module powers up to 8 *neMESYS 290N* low-pressure syringe pump modules and can be controlled by a PC via an integrated USB interface. The pumps are daisy chained to the base module via serial ports with a termination plug at the terminal port (see Figure 1).

As an alternative to the low-pressure pumps, which exert a maximum force of 290 N, the manufacturer also offers much more powerful pump modules that deliver 1000 N, 2600 N, and even up to 7000 N (*neMESYS 1000N*, *neMESYS 2600N*, and *neMESYS 7000N*, respectively*)*, which may be used to deliver more viscous stimuli. The power supplied through the BASE module determines the type and number of pumps that can be operated simultaneously. For example, the *BASE 120* module can drive up to 8 low-pressure pumps, while the *BASE 600* can operate 41.

The neMESYS syringe pumps can create uniform, pulsation-free fluid streams and accurately dose fluids down to the nanoliter range. Pumps can be used in *sequential* order (one after the other) and in *parallel* (multiple pumps acting simultaneously). *Continuous* flow can be generated by emptying and filling two or more syringes in alternating order (i.e., one set of pumps is dispensing from filled syringes while another set refills the previously emptied syringes).

The functionality of the pump system can be widened further by a large assortment of supplementary *Qmix* modules, including input/output modules (which can be used for sending and receiving trigger signals), valve, heating, pressure monitoring, spectrometric, and camera modules (Cetoni GmbH, Korbussen, Germany).

Syringes

We installed 50 mL high-precision glass syringes (SETonic GmbH, Ilmenau, Germany) in each pump to reduce the frequency at which syringe refill was necessary during operation (Figure 1). Smaller syringes (from 10 µL to 50 mL) can be installed as needed and offer an effective way of increasing the pressure exerted by the pumps[*]. This can be particularly useful when working with high-viscosity fluids. Alternatively, the manufacturer offers syringes made of stainless steel to handle even higher pressures; these syringes, however, do not

[*] $p = F/A$, with p: pressure produced by the pump, F = const.: force applied by the pump, A = surface area of the syringe plunger, which directly depends on the syringe diameter. With decreasing syringe diameter, the exerted pressure can increase while the applied force remains constant.

allow for visual check of fill level and identification of potentially trapped air bubbles. It should be noted that the universal syringe connectors on the neMESYS syringe pumps do not limit the user to one specific syringe manufacturer but allow to select from numerous types of syringes from different producers.

System clamp

The base and pump modules were mounted in a so-called *system clamp,* a metal bracket which positions the pumps and installed syringes in an upright position (Figure 1). The vertical alignment is not required by the manufacturer, but it ensures that air bubbles float up and accumulate at the top of the syringes, from where they can be easily removed by partially emptying the syringes.

Computer-controlled valves

Each syringe was connected to a computer-controlled 3/2-way solenoid valve with 3 ports (Cetoni GmbH, Korbussen, Germany; see Figure 1). In this type of valve, one of the three ports is permanently opened. This port is connected to the syringe. The other two ports act in a reciprocal manner: when one is opened, the other is automatically closed. The valve state is controlled by a computer, allowing the user to switch the opening of the ports as needed. A typical setup would be one port connected to the stimulus reservoir for stimulus refill and to use the other port to deliver the stimulus to the participant by dispensing from the syringe. This setup additionally enables the experimenter to refill the syringes between consecutive stimulus deliveries. For convenience, we will henceforth name the port that is connected to the stimulus reservoir the inlet port, while the port that leads to the mouthpiece will be referred to as the outlet port.

Tubing

The inlet port of each valve was connected to a stimulus reservoir via 1 m ETFE[†] tubing (1/16" inner diameter, 1/8" outer diameter; Techlab, Braunschweig, Germany), and each outlet port was connected to a custom-made mouthpiece via 3.2 m tubing. Syringes were attached to the solenoid valves via 0.1 m tubing.

Tubing connections

All tubing connections were established via flangeless fittings and their accompanying ferrules (IDEX Health & Science LLC, Oak Harbor, WA/USA) to ensure tight and leak-proof links.

[†] Ethylene tetrafluoroethylene, a derivative of PTFE, commonly known by its brand name, Teflon.

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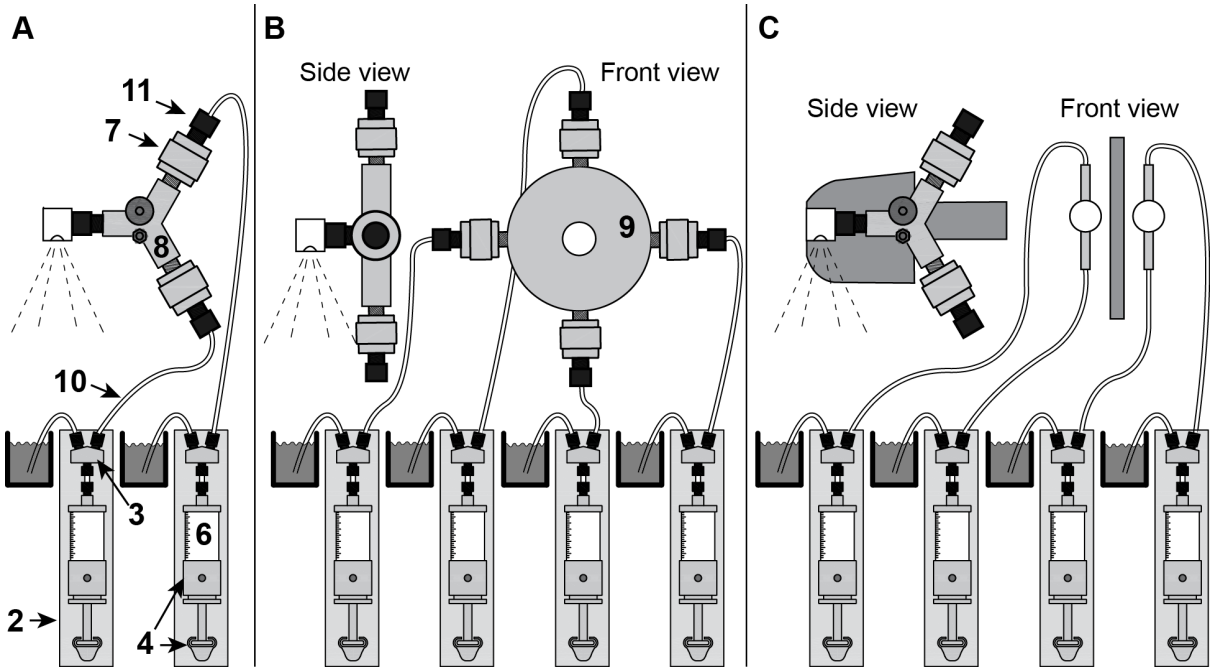

Figure 2. Schematics of the gustometer setup. (2) pump module, (3) 3/2 solenoid valve, (4) syringe holder and piston holder, (6) glass syringe, (7) inlet check valve, (8) Y-connector, (9) 5-way manifold, (10) tubing, (11) fitting. (A) Setup for up to two stimuli. (B) Setup for up to 4 stimuli. (C) Setup for lateralized stimulation with up to two stimuli on each side of the tongue. A photograph of the mouthpiece is also shown in Figure 5A.

Mouthpiece

To deliver the stimuli onto the tongue, we used a mouthpiece with a spray head attached to the outlet of a manifold via a short stub of tubing. However, the mouthpiece itself does not require the connection of any spray head, but rather allows the user to choose an outlet that is best suited to the experimental needs. For example, the stub of tubing can be attached to a custom-made anatomically shaped structure, or participants can even hold the stub of tubing gently between their teeth. There is virtually no limitation as long as the outlet can be connected with the tubing in a leak-proof manner. We opted for a spray head because it atomizes the liquid and evenly distributes it to a large surface area of the tongue.

More syringes, each supplied by a different stimulus reservoir, can be used in a multi-stimulus setup; here, the tubing from *n* pumps would terminate in an *n+1*-way manifold with an inlet check valve (IDEX Health & Science LLC, Rohnert Park, CA/USA) for each tube to prevent backflow of the liquids, and an outlet of the user's choice, e.g. a spray head (Burghart, Wedel, Germany). The system further allows to attach multiple mouthpieces at

once, which may be used to stimulate the left and the right side of the tongue independently. We used this setup in combination with a separator that was placed along the midline of the tongue in a lateralized stimulation experiment (see the section of that name, and Figures 2C and 5A).

Control software

The pump manufacturer, Cetoni, provides the proprietary Qmix Elements software that allows the user to control the pump system via a powerful graphical interface. This software allows for easy basic control of the system and is even partly automatable. Yet, full integration into existing laboratory processes and experimental procedures often requires a much higher degree of flexibility. For example, the researcher may wish to align pump sequences with the presentation of visual stimuli, or to synchronize pumping with laboratory hardware (e.g., EEG amplifiers, MR scanners) via TTL pulses.

Fortunately, Cetoni also offers a Qmix software development kit (Qmix SDK). It exposes the full functionality of the pump system via a well-documented application programming interface (API) in the C++ programming language. However, this language is not widely used in neuroscience and psychology labs – mostly because it is difficult to master without a background in computer science. We therefore decided to create a comprehensive and easy-to-use Python package, called *pyqmix*, that maps Python function calls to their corresponding C++ counterparts in the SDK. Over the past years, the Python programming language has progressively managed to establish itself as a free competitor to proprietary software like Matlab for neuroscientific experimental control, thanks to powerful packages like PsychoPy (Peirce, 2009) and expyriment (Krause & Lindemann, 2014). As a full-grown programming language, Python offers a plethora of possibilities to present and manipulate stimuli, record and process participants' responses, and to control a large variety of laboratory hardware. *pyqmix* is free and open-source software, released under the GNU General Public License (GPL). This means that the source code is available free of charge and it can be modified by the user. The following code snippet demonstrates how to fill a pump at a flow rate of 0.5 mL/s, followed by dispensing 1 mL at a flow rate of 1 mL/s.

```
pump.fill(flow_rate=0.5, wait_until_done=True)
pump.dispense(volume=1, flow_rate=1)
```

For more complex examples, please refer to Appendices A and B. Full documentation including installation instructions is available at pyqmix.readthedocs.org.

Web interface

To further simplify operation of the pumps, we implemented a remote-control interface that can be accessed via a web browser (Figure 3). This software, *pyqmix-web,* interfaces with *pyqmix* either on a local or remote computer and allows to perform common tasks in a straightforward way, including pump calibration (reference move), and filling, emptying and rinsing of the syringes. Additionally, it implements a method for the removal of bubbles that often get trapped in the syringes during initial filling. The web application guides the user through all required steps of these procedures. No prior programming knowledge is required to use the app. We provide a self-contained executable at https://github.com/psyfood/pyqmix-web. No installation is required, as the executable contains all required dependencies, including Python and *pyqmix*. Like *pyqmix,* the software is free and open-source (GPL-licensed).

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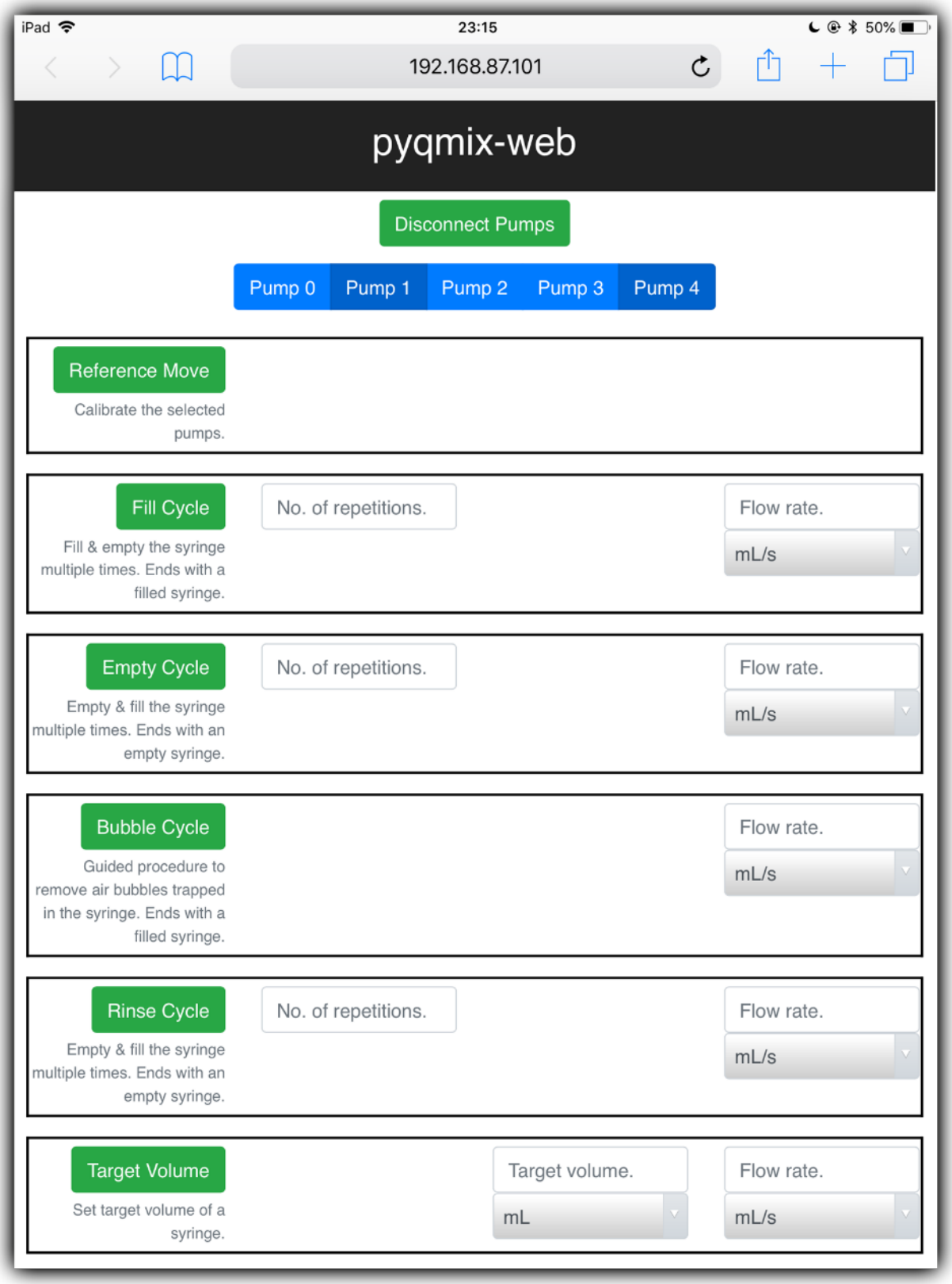

Figure 3. Screenshot of the *pyqmix-web* interface running on an iPad. Five pumps have been detected in the gustometer. The user has selected Pumps 1 and 4 and may now calibrate the pumps by a executing a reference move, set the target volume or initiate pump activities like filling, emptying, rinsing, or the bubble cycle to remove excess air from the syringes.

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Temporal properties

To verify the suitability of the gustometer for experiments that demand precisely timed liquid stimulation with steep stimulus onset flanks, we measured two key temporal properties of stimulus delivery (onset delay and rise time) through a spray head.

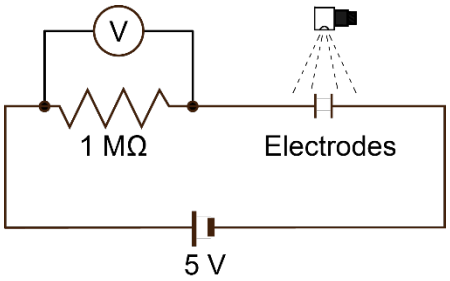

Figure 4. Electrical circuit used for the measurement of onset delay and rise time of stimulus delivery.

An electrical circuit was used to detect stimulus onset delay and rise time (Figure 4). For this, two electrodes, spaced 5 mm apart, were placed on a surface 5 mm below the spray head of the mouthpiece illustrated in Figure 2A. The surface was tilted to 45°, allowing the liquid to drip off (see Kelling & Halpern, 1986 for a similar procedure). A National Instruments USB 6212 data acquisition board (National Instruments, Austin, TX/USA) provided a +5 V direct current voltage source to the circuitry and was used to register changes in electrical conductivity between the electrodes as the space between them was covered with liquid. The electrical conductivity between the electrodes was indirectly measured as the potential difference (in volts) across a 1 MΩ resistor at a sampling rate of 2000 Hz.

Two 50 mL syringes were attached to pumps and filled with low concentration salt water (8.556 mM sodium chloride, i.e. 0.5 g/L dissolved in de-ionized (DI) water). The two pumps were set to dispense 1 mL at 1 mL/s in alternating order. Inter-trial-interval was 10 s to allow the surface between the electrodes to dry. The measurement of electrical conductivity between the electrodes was initiated as the pump was triggered to dispense and ended after 2.5 s. Following each dispense, the valve was switched from outlet to inlet position for ensure sharp offsets. The procedure was repeated until both syringes were almost emptied, resulting in a total of 49 trials per pump.

The first 4 trials served to wet the electrodes. We determined rise time and onset delay for each of the remaining 45 trials. Recordings were baseline-corrected by subtracting the mean voltage from 10 to 40 ms after pump initiation. Onset delay was defined as the time from

pump initiation until 50% of the maximum voltage in that trial was reached. Rise time was defined as the time between 25% and 75% of the maximum voltage.

The measurement data is available at Zenodo (https://doi.org/10.5281/zenodo.1313034).

Dosage precision and accuracy

To verify that the gustometer delivers the desired volume, we collected 10 samples of putative 1 mL dosages of distilled water at ambient temperature from one pump and determined the mass using a high-precision laboratory weighing scale (precision = 1 mg; Kern 572-30, Kern & Sohn GmbH, Balingen, Germany). The collected volume was determined based on the density of water at 20 °C, which is 0.998 g/mL.

Lateralized stimulation experiment

To test whether the hemitongues could be independently stimulated, which would facilitate studies of the lateralization of the gustatory processing pathway, and to show that the proposed stimulation elicits an evoked electrophysiological response, we conducted an experiment using two parallel mouthpieces (Figures 2C and 5).

Participants.

18 participants (age mean +/- standard deviation: 28.8 +/- 4.4 years; 13 women; 1 left-handed) took part in the study. Data from two participants (both 26 years old, female, right-handed) performing the experiment while EEG was recorded are included as proof of concept. Participants were instructed to refrain from eating, drinking (except water), smoking, and brushing teeth for at least 30 min prior to the experiment to avoid any uncontrolled influences on orosensory perception (see Hummel, Genow, & Landis, 2010; Jacquin-Piques et al., 2015; Iannilli, Broy, Kunz, & Hummel, 2017 for similar procedures). The study was approved by the ethical board of the German Society for Psychology (DGPs) and conformed to the Declaration of Helsinki. All participants gave written and oral informed consent prior to the study and received monetary compensation.

Stimuli and Apparatus.

A cushioned headrest was placed in front of a TFT (thin film transistor) computer monitor at a distance of 60 cm. A mouthpiece was attached to the headrest; it consisted of two 3-way manifolds (2 inlets, 1 outlet) placed on the left and right side of a vertical, 5 mm wide plastic separator (see Figures 5A and 2C). The separator was to be positioned along the midline of the tongue and served to enable stimulation of the left and right side of the tongue

independently, without stimuli crossing sides. Each inlet port was connected to a dedicated syringe: deionized water was supplied to one port (serving as rinse and as *touch* stimuli), and a salty solution (0.342 M sodium chloride, i.e. 20 g/L, dissolved in DI water) was supplied to the other port (serving as *taste* stimuli; Figure 2C). Spray heads were connected to the outlets of the manifolds. The pump system was placed outside of a sound-attenuated experimental booth.

Procedure.

Participants were seated in the sound-attenuated booth, instructed to rest their forehead on the headrest and to protrude the tongue such that the anterior part of the tongue was held against the separator, spatially separating the tongue at the midline, with the spray heads hovering approximately 5 mm above the tongue. The stimuli were sprayed onto the tongue and consecutively dripped off into a bowl to avoid swallowing.

Participants performed two interleaved tasks, a *touch* and a *taste* task. At the beginning of each trial, the type of the upcoming task was displayed on the computer monitor for 2 s. Then, a fixation cross appeared in the center of the screen. After a random duration of 1 to 2 seconds (selected from a uniform distribution), stimulus delivery was initiated for a duration of 1 s at a flow rate of 1 mL/s. In *touch* trials, water was delivered to either the left, the right, or both hemitongues; in *taste* trials, salty solution was delivered to both hemitongues, or only to the left or the right hemitongue while water was sprayed onto the other; see Figures 5 B and C. This was done to ensure a similar tactile stimulation across both sides of the tongue during *taste* trials. To ensure a sharp stimulus offset, the outlet ports of the valves were closed immediately after the pumps had stopped delivering the stimulus. Three seconds after stimulus offset, the computer monitor prompted participants to report the locus of stimulation (*touch* trials: side of tactile stimulation; *taste* trials: side of salty stimulation) by pressing either of three buttons on a keyboard (left arrow – left side, right arrow – right side, down arrow – both sides). The response hand changed halfway through the experimental session; the starting hand was counterbalanced across participants. A blank screen was presented immediately after registering a response or after 5 seconds if no response was provided and stayed until the next trial started (for a minimum of 4 s in *touch* and 7 s in *taste* trials). The hemitongues were rinsed with 3 mL of water each during this inter-trial interval (ITI) following *taste* trials. The inter-stimulus interval (ISI) between a *touch* and the consecutive *taste* stimulus was 11.0–13.0 s, and the ISI between a *taste* and the consecutive *touch* stimulus was 14–16.9 s. Water syringes were refilled during the ISI.

In total, 180 *touch* and 180 *taste* trials were presented in a pseudo-randomized order such that the same condition would not occur on more than two consecutive trials within each task. The experiment was split into 6 consecutive blocks with an equal number of trials (i.e., 60 trials per block). Taste trials were recorded for use in another study and are only reported here for the two EEG participants.

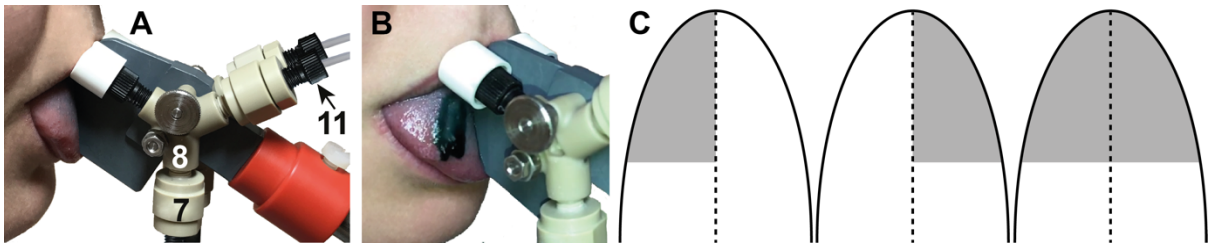

Figure 5. (A) The mouthpiece used in the experiment. Lateralized stimulation is achieved through two separate spray heads placed on either side of a plastic separator (see also Figure 2C for the schematics of the setup). (B) Demonstration of the lateralized stimulation locus using a colored liquid. Representative still image extracted from a high-speed video recording (240 frames per second; iPhone SE, Apple Inc., Cupertino, CA/USA). (C) Illustration of the experimental conditions: stimulation of the left side, right side, and both sides of the tongue.

To ascertain that stimulus delivery allowed the recording of evoked potentials, we measured the electrophysiological response in the lateralized stimulation experiment using an actiCHamp amplifier system with 64 Ag/AgCl active channels positioned according to the extended 10-10 system (Brain Products GmbH, Munich, Germany). EEG data was sampled at 500 Hz and passed through an analog 0.01 Hz high-pass and a 200 Hz low-pass filter using PyCorder (Brain Vision LLC, Morrisville, NC, USA). We sent a +5 V trigger pulse to the EEG amplifier via the National Instruments data acquisition board when the syringe pumps were started, marking the pump onset in the continuously recorded EEG stream.

Data analysis – Touch localization

For each participant and condition, the proportions of *left*, *right*, and *both* responses were calculated. The results were then averaged across participants, yielding a grand mean confusion matrix. Statistical significance of the responses was evaluated by averaging the proportions of correct responses for each participant and comparing the result against chance level (33.3%) using a one-sample t-test. Trials without a response were omitted from the analysis.

Data analysis – Evoked responses

EEG data was analyzed offline using MNE (version 0.16; Gramfort et al., 2013) in Python (version 3.6.6). First, we removed drifts in the data by linear detrending and then applied a zero-phase high-pass and low-pass hamming-windowed FIR filter with a cut-off at 0.25 Hz and 12 Hz, respectively (transition width: 0.25 Hz for the high-pass filter and 3 Hz for the low-pass). The stimulus onset trigger times were shifted according to the stimulus onset delay which was determined as described in the *Temporal properties* section, and data was segmented into epochs ranging from -0.2 to 1.0 s relative to stimulus onset.

Based on visual inspection, we interpolated excessively noisy channels and channels that showed large non-stereotypical artifacts in individual epochs. Epochs with unique, non-stereotypical artifacts were excluded (<2% of epochs removed). Stereotypical artifacts, such as ocular, cardiac, and muscle activity were removed by rejecting their corresponding independent components estimated from an ICA decomposition (FastICA; Hyvarinen, 1999) of data generated from an equivalent run of the preprocessing steps described above, except for the FIR filtering step, in which cut-off frequencies of 1 Hz and 40 Hz were used instead.

Lastly, the data was re-referenced to the average of all channels; the mean of the 200 ms pre-stimulus baseline was subtracted from the signal; and individual channels were interpolated in epochs that had been marked as problematic by Autoreject (Jas, Engemann, Bekhti, Raimondo, & Gramfort, 2017). All t*ouch* and *taste* trials, respectively, were averaged within participants. The reference-independent amount of electrical activity was calculated as the spatial standard deviation across channels at every time point (global field power, GFP; Lehmann & Skrandies, 1980).

Results and Discussion

Temporal properties

Onset Delay

Exact control of stimulus onset delay is paramount to allow temporally precise response time measures and to take full advantage of the high temporal precision of electrophysiological recordings (EEG or MEG). Stimuli were delivered with an onset delay of 44.1 ms +/- 2.6 ms (mean +/- standard deviation) for one pump and 48.3 ms +/- 2.9 ms for the other (Figure 6).

The near-constant onset delay suggests that the gustometer is indeed suitable for use in electrophysiological studies and obviates the need for complex online detection of stimulus onset.

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Rise Time

Likewise, it is important that the gustometer delivers stimuli with a short rise time to activate a large number of receptors in the targeted region simultaneously. The stimulus pulses delivered by our gustometer had a rise time of less than 2 ms. Specifically, measurements showed rise times of 1.9 ms +/- 1.6 ms (mean +/- standard deviation) for one pump and 1.9 ms +/- 1.2 ms for the other. The device is able to deliver stimuli with rise times as short as previously reported gustometers, albeit the authors used differing definitions of rise time or did not specify an exact definition at all (< 15 ms, Crouzet et al., 2015; < 20 ms, Kobal, 1985 and Kobayakawa et al., 1996; < 50 ms, Iannilli et al., 2015).

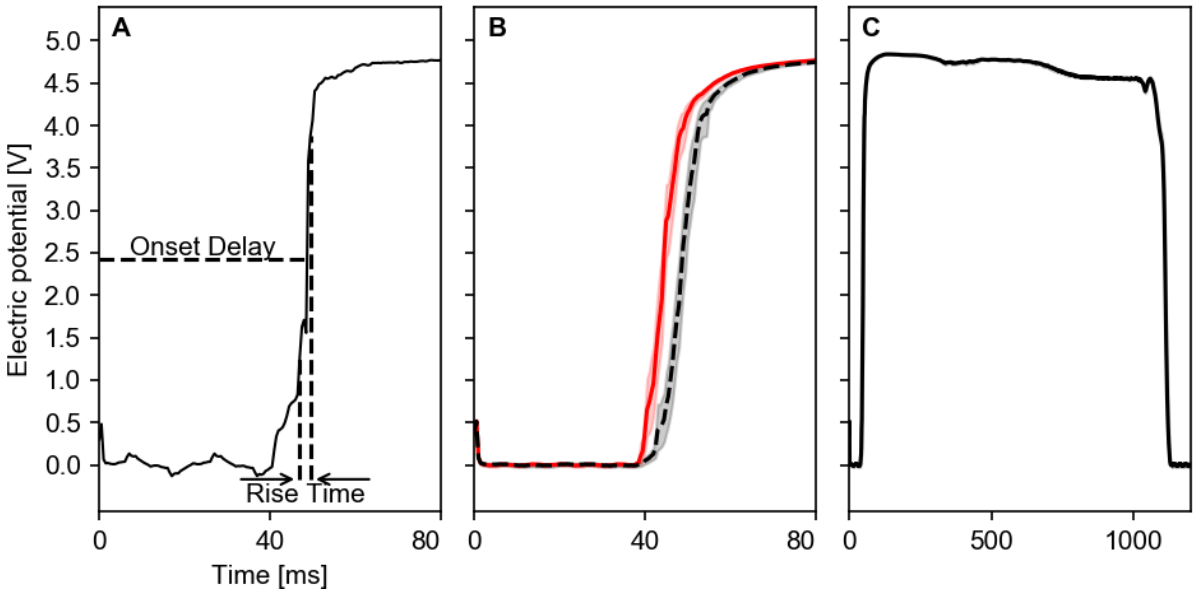

Figure 6. Onset delay and rise time measurements. Pumping was initiated at time point zero and stopped after 1000 ms, at which time the valves were switched to the inlet position. We measured the electric potential between two electrodes positioned below the spray head. (A) Sample measurement from one pump. The oscillations before approx. 40 ms are 50 Hz line noise artifacts in the measurement circuit. (B) Average signal onsets across all measurements for two pumps. (C) Average signal across all measurements for one pump. The shaded areas depict the 95% confidence intervals, derived via bootstrapping (1000 resamples).

Dosage precision and accuracy

Dosage accuracy was high as the dosed volumes only deviated slightly from the desired volume of 1 mL. Specifically, we measured the average dispensed volume to be 0.98 mL, suggesting a volume bias of approx. 2%. Precision was excellent, with a standard deviation of just 0.01 mL. Overall, the data show that the gustometer reliably delivers the desired

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volumes, demonstrating that the amount of delivered stimulus material can be precisely controlled.

Lateralized stimulation experiment

Touch localization

Identification accuracy of the locus of stimulation was high (91%) and significantly exceeded chance level (t_{17}=28.33, p<0.001), corroborating that the liquids did not cross the separator (see Table 2 for a confusion matrix). On average, participants responded to > 98% of trials.

Table 2. Confusion matrix of behavioral responses from the touch localization task. Values in parentheses are standard deviations.

	Proportion of Responses (in %)		
Stimulus Location	Left	Both	Right
Left	96.0 (4.5)	3.4 (4.2)	0.7 (1.2)
Both	8.9 (13.4)	85.5 (19.1)	5.6 (8.4)
Right	2.3 (4.5)	5.3 (4.9)	92.4 (7.3)

Evoked responses

Two participants correctly reported the side of touch stimulation in 99% and 95% of trials, respectively (Participant 1 and 2). For taste stimulation, the proportions of correct localizations were 90% and 83%. Mean global field power after *touch* and *taste* stimulation was derived for both participants; the traces are shown in Figure 7. *Taste* signal amplitudes exceeded the *touch* amplitudes for most time points. These data show that the device is indeed suitable for stimulation in EEG experiments, as it allows to successfully elicit evoked potentials.

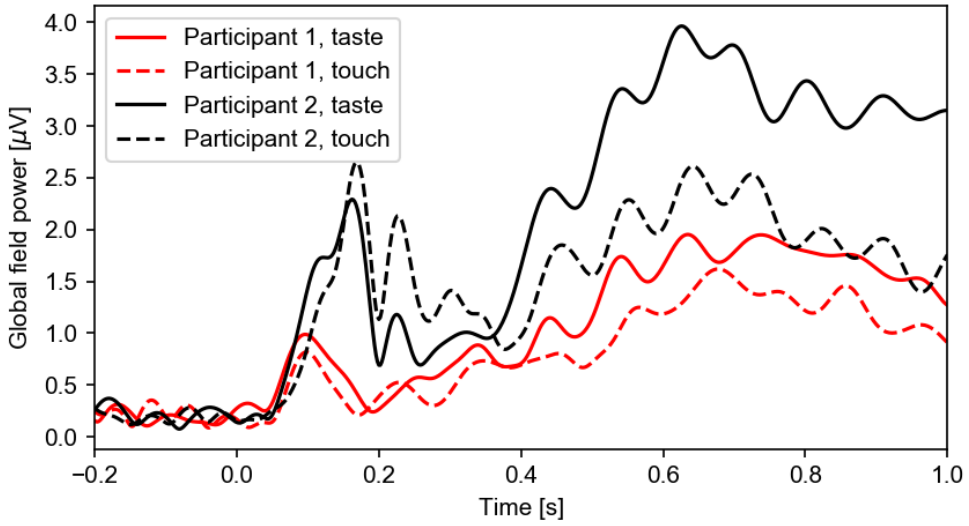

Figure 7. Mean global field power (GFP) for *touch* and *taste* stimulations. For both participants, the *taste* signal exceeds the *touch* signal over large periods of time.

Possible extensions and design alterations

The modularity of the gustometer makes it possible to extend and alter its functionality in many ways.

Greater number of stimuli. The gustometer can easily be extended to include more stimuli by adding pumps and adjusting the manifold in the mouthpiece.

Type of mouthpiece. The gustometer does not impose the use of a mouthpiece of any specific type or shape; in fact, it can be used without any mouthpiece at all. The manner and site of stimulus delivery can therefore be fully adjusted to the experimenter's needs, be it to stimulate the tongue uni- or bilaterally; to spray; or to simply let the stimulus flow onto the tongue.

Controlled stimulus temperature. Cetoni offers heating and cooling modules. Warming stimuli to tongue temperature can effectively avoid lingual temperature sensations. Alterations of stimulus temperature may also be used to study the effect of temperature differences on flavor perception.

Continuous stimulation. In the experiments presented here, stimuli were delivered without preceding tactile stimulation, evoking both a gustatory and tactile sensation much like in everyday eating situations. However, stimuli may also be embedded in an ongoing flow of "background rinse", avoiding concomitant onset of gustatory and tactile sensation.

Electric triggers. The gustometer can be equipped with an input/output module (Qmix IO-B), which allows generating and receiving electrical trigger signals to interface with other laboratory devices, including EEG and fMRI systems.

Viscous stimuli. The gustometer can deliver viscous stimuli by employing the mid-pressure variant of the neMESYS syringe pump system (Cetoni GmbH, Korbussen, Germany) or alternatively, by using syringes with smaller diameter (as the generated pressure increases inversely proportionally with the cross-section area of the syringe).

Advantages of our gustometer

The main advantages of our gustometer are presented in the following section.

Precision & accuracy. The gustometer precisely doses stimuli with practically constant onset delay, steep stimulus onset flanks, and accurate volume. These properties represent essential requirements of most behavioral, psychophysiological, and neuroimaging experiments, and ensure stimulation replicability across trials and participants.

Automation. The gustometer is computer-controlled. Our free and open-source Python-based software package allows the user to control the syringes within any experiment programmed in Python, e.g. PsychoPy (Peirce, 2009) and expyriment (Krause & Lindemann, 2014). This includes the refill of the syringes during a running experiment, which allows the delivery of large stimulus volumes and/or large number of stimuli, and even continuous stimulation when using two pumps interchangeably.

Modularity. The gustometer offers fully user-customizable setups. The experimenter can connect a variable number of mouthpieces and modules depending on the experimental requirements at hand (for further information please refer to the previous section: *Possible extensions and design alterations*).

Hygiene. Since all components of the gustometer are commercially available "off the shelf" and accessible, they can be easily replaced, offering excellent hygiene and minimizing tastant contamination between experiments.

Portability. The gustometer is portable due its comparably small dimensions and also because it does not require compressed air or other fixed installations. It can therefore easily be set up in different experimental locations and, if installed on a wheeled table or cart, it can even be made mobile.

Low initial costs. The user can opt to purchase a smaller version of the gustometer with only a few pumps at first, and expand the gustometer later as more funds become available or experimental complexity increases.

Best practices

During construction and testing of the gustometer, we discovered several potential pitfalls that can be easily averted. We therefore compiled a collection of *best practices* to ensure reliable, precise and accurate operation.

Avoid air bubbles. Upon initial filling, air from the inlet tubing ends up in the syringes and must be removed before the experiment commences; otherwise, precision and accuracy of stimulus delivery are compromised by the compressibility of the air in the syringe. A viable method to remove air from the syringes is filling and dispensing liquid repeatedly while the syringe is in an upright position. This procedure, at the same time, removes air from the outlet tubing. Once syringes and tubing are completely filled with liquid, the gustometer is ready for use in an experiment. There will be no further accumulation of air even during refilling, as long as the inlet tubes stay submerged in liquid and the refill flow is sufficiently slow.

Dispense after filling. We observed that (re)filling of the syringes altered the onset delay of the first few subsequent dispense operations. This bias can be effectively avoided by dispensing a small volume after each filling procedure, for example as a part of a tongue rinse, a break, or during any other period of the experiment in which the onset delay is not critical.

Mount syringes tightly. To ensure temporal precision of the gustometer, the position of the syringe piston holder must be checked and possibly adjusted every time a syringe is attached to a pump. The holder should fit very tightly. Please also consider that there may be small manufacturing differences, even between syringes of the same size.

Select the appropriate pump modules. If the force required to move the syringe piston exceeds the maximum force the can be produced by the pump module, the motor stops and the pump switches into a *fault state*, meaning it will remain non-operational reset. It is therefore important to select the pump modules according to the specific experimental requirements. Low-pressure pumps are ideal for delivering liquids with low viscosity (like water) at moderate flow rates through relatively short tubes. If the experimenter desires to use stimuli with higher viscosity (e.g., oil), or to deliver liquids at high flow rates and through long tubes, mid-pressure pumps are highly recommended.

Keep the tubing short. Each centimeter of tubing adds additional friction to the liquid flow, potentially influencing onset delay, rise time, and pump force required. Long inlet tubes limit the flow rate at which the syringe can be reliably filled without producing air bubbles. Therefore, always ensure not to use longer tubes than absolutely necessary.

Close valves after stimulation. Temporally precise stimulus offset can be achieved by closing the valve outlet immediately after pumping has stopped. Potential excess pressure in the system will force the remainder of the stimulus back into the stimulus reservoir instead.

Rinse after use. Pumps and tubing should be rinsed after each recording session. As the pumps can be programmed to fill and dispense automatically, it is possible to automate this procedure and conduct a thorough rinse of the system, e.g. with ethanol or distilled water.

Revise temporal properties. There are several factors which may influence rise time and onset delay of the gustometer, including tubing length, syringe size, viscosity of the liquids, flow rate, dispensed volume, distance between tongue and mouthpiece, etc. It is therefore important to note that rise time and onset delay are setup-specific and must be reevaluated whenever modifying central parts of the system.

Avoid online mixing. When attaching multiple syringes to the same manifold or mouth piece, it might be tempting to mix stimuli “online” by dispensing from two or more syringes simultaneously. Depending on the specific type of mouth piece used, this procedure might lead to unpredictable effects as there is no way to ensure that the stimuli are being mixed properly before reaching the tongue. We therefore suggest to avoid online mixing if possible, and to test the success of the mixing procedure very carefully in cases where mixing is unavoidable.

Conclusion

We presented a gustometer that meets the demands of behavioral, electrophysiological, and hemodynamic investigations. Our measurements showed that stimulus timing and dosage volume were precise and accurate. Stimuli were delivered with steep onset flanks. We further demonstrated how the device can be used in lateralized behavioral testing, delivering stimuli to the left and right hemitongues independently. While other researchers have used cheaper syringe pump systems than the one presented here in electrophysiological studies (e.g. Andersen et al., 2018; Franken, Huijding, Nijs, & van Strien, 2011), the high-precision pumps of our gustometer provide several advantages. Delivering higher flow rates, they

enable the experimenter to produce a fine spray without requiring an additional supply of compressed air. The fully disclosed design and freely available control software (including the publicly available source code) make adjustments and design alterations for different experimental requirements easy. This versatility is further enhanced through the ability to add additional hardware modules to the system, e.g. for digital I/O and stimulus heating. We hope that the great flexibility of the gustometer, its simple construction and operation, and the relatively low entry price will encourage more scientists to join the community of taste research and enrich this widely unexplored field with new and exciting discoveries.

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The gustometer was designed and the behavioral and EEG data was collected at the German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE). The timing measurement were conducted, all data was analyzed, and the manuscript was written after KO and RH had moved to Research Center Jülich.

The authors declare no conflict of interest.

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Appendix A: pyqmix usage example with one pump

The following code snippet illustrates a basic use case of *pyqmix* to initialize a pump, fill it, and dispense the liquid in ten 1 mL aliquots at a flow rate of 1 mL/s with an inter-stimulus interval of approx. 2 s.

```
#!/usr/bin/env python
# -*- coding: utf-8 -*-

"""
This simple usage example demonstrates how to initialize the pump system, fill
one syringe, and dispense a small volume multiple times.
"""

from pyqmix import QmixBus, QmixPump, config
import time

# Qmix device configuration.
config.set_qmix_config('qmix_config')

# Initialize the connection to the pump system.
bus = QmixBus()

# Initialize the first connected pump and perform a calibration move.
# Program execution is halted until the move is completed.
pump = QmixPump(index=0)
pump.calibrate(wait_until_done=True)

# Set pump and syringe parameters.
pump.set_flow_unit(prefix='milli', volume_unit='litres',
                   time_unit='per_second')
pump.set_volume_unit(prefix='milli', unit='litres')
pump.set_syringe_params_by_type(syringe_type='50 mL glass')

msg = ('The system is now calibrated. Please insert the syringe.\n\n'
       'Press RETURN when done.')
input(msg)

# Fill the syringe at a flow rate of 0.3 mL/s, and halt program execution
# until the filling is completed.
pump.generate_flow(-0.3, wait_until_done=True)

msg = ('The syringe is now filled. To start the experimental procedure, '
       'press RETURN.')
input(msg)

# Dispense 1 mL at a flow rate of 1 mL/s ten times, with a break of approx.
# 2 seconds between dispenses.
for i in range(10):
    pump.dispense(volume=1, flow_rate=1, wait_until_done=True)
    time.sleep(2)

msg = 'Stimulation is over. Press RETURN to quit.'
input(msg)
```

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Appendix B: pyqmix usage example with two pumps

```
#!/usr/bin/env python

"""
This usage example demonstrates how to initialize the pump system, fill
one syringe with water and one with a salty solution, and embed the
salty stimulation in-between a continuous stream of water.
"""

from pyqmix import QmixBus, QmixPump, config
from time import sleep

# Qmix device configuration.
config.set_qmix_config('qmix_config')

# Flow and volume units and dimensions of the syringes.
flow_unit = dict(prefix='milli',
                 volume_unit='litres',
                 time_unit='per_second')

volume_unit = dict(prefix='milli', unit='litres')

# The actual flow rates to use. Units as specified above (mL/s).
flow_rate = dict(fill=-0.3,
                 water=1.0,
                 salty=1.0)

# Initialize the connection to the pump system.
bus = QmixBus()

# Initialize pumps.
#
# We will later fill the syringe in the first pump with water and the syringe
# in the second pump with a salty aqueous solution.
pump = dict(water=QmixPump(index=0),
            salty=QmixPump(index=1))

for p_name, p in pump.items():
    p.set_flow_unit(**flow_unit)
    p.set_volume_unit(**volume_unit)
    p.set_syringe_params_by_type(syringe_type='50 mL glass')

# Fill the syringes and halt program execution until the filling is completed.
pump['water'].generate_flow(flow_rate['fill'])
pump['salty'].generate_flow(flow_rate['fill'], wait_until_done=True)

msg = ('The syringes are now filled. To start the experimental procedure, '
       'press RETURN.')
input(msg)

# Run a dispense cycle (water – salty – water) 10 times, with a break of
# approx. 5 s between stimulations.
for i in range(10):
    pump['water'].dispense(volume=1, flow_rate=flow_rate['water'],
                           wait_until_done=True)
    pump['salty'].dispense(volume=1, flow_rate=flow_rate['salty'],
                           wait_until_done=True)
    pump['water'].dispense(volume=2, flow_rate=flow_rate['water'],
                           wait_until_done=True)
    sleep(5)

msg = 'Stimulation is over. Press RETURN to quit.'
input(msg)
```

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