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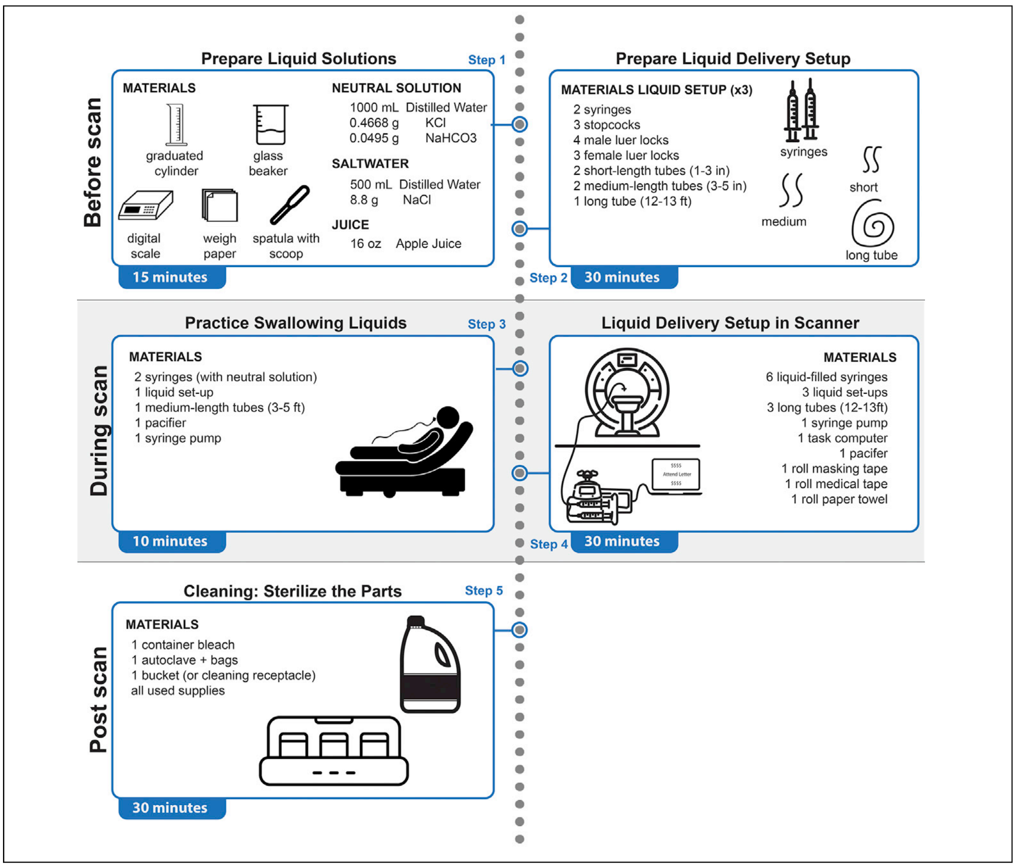
Protocol

An fMRI protocol for administering liquid incentives to human participants



This protocol describes the materials and approaches for administering liquid incentives to human participants during fMRI scanning. We first describe preparation of the liquid solutions (e.g., neutral solution and saltwater) and liquid delivery setups. We then detail steps to connect the setups to the computer-controlled syringe pump in the MRI control room, followed by procedures for testing the syringe pump dispensing a liquid bolus during the task. Description of custom software and required adapters for implementing the liquid setup are included.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Detailed protocol to administer liquid incentives to humans during fMRI scanning

Computer-controlled syringe pump delivers liquid to participants with high precision

Recipes for neutral and saltwater solution for liquid delivery during fMRI task

Descriptions of custom software and required adaptors for implementing liquid setup

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Protocol

An fMRI protocol for administering liquid incentives to human participants

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SUMMARY

This protocol describes the materials and approaches for administering liquid incentives to human participants during fMRI scanning. We first describe preparation of the liquid solutions (e.g., neutral solution and saltwater) and liquid delivery setups. We then detail steps to connect the setups to the computer-controlled syringe pump in the MRI control room, followed by procedures for testing the syringe pump dispensing a liquid bolus during the task. Description of custom software and required adapters for implementing the liquid setup are included.
For complete details on the use and execution of this protocol, please refer to Yee et al. (2021).

BEFORE YOU BEGIN

The protocol below describes the specific steps for administering liquids (e.g., juice, neutral solution, saltwater solution) to human participants in a task-based fMRI study examining the neural mechanisms underlying the bundling of monetary and liquid incentives (Yee et al., 2021). This protocol provides information regarding how the tubes should be connected to a controlled syringe pump to provide three different types of liquid as feedback to correct responses during the behavioral experiment. This protocol also provides the MATLAB code to control the pump based on behavioral responses from the participant performing the main task. For detailed information of the main behavioral study, please refer to the referenced publication: Yee et al., Journal of Neuroscience (2021).

All study procedures detailed in the Step-by-step methods details can be performed on the day of the scan. However, both the Preparation of the Liquid Solutions and the Prepare Liquid Delivery Setup can be completed up to a day in advance, if desired (see more on storage of the liquid solutions below).

Institutional permissions

All participants in the original study (n=51; 25 females; 18–38 years; mean age = 25.1, sd age = 4.8) provided written consent approved by the Washington University Institutional Review Board, and received payment for their participation plus additional earnings of up to 15 dollars based on task performance. Those who aim to use the protocol in studies with human subjects must acquire permission from their relevant institution.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sodium Bicarbonate ($NaHCO_3$)	Fisher Scientific	S631-3
Potassium Chloride (KCl)	Fisher Scientific	P333-500

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sodium Chloride (NaCl) [non-iodized]	Morton	N/A
Distilled Water	N/A	N/A
Apple Juice	Mott's	N/A
Experimental models: Organisms/strains		
Human subjects (n=51, 25 female, 18–38 years)	Yee et al. (2021)	N/A
Software and algorithms		
MATLAB (MathWorks)	https://www.mathworks.com	Version: 2016b
Psychtoolbox	http://psychtoolbox.org	Version: 3.0.12
Deposited data		
Datasets and code	Yee et al. (2021)	*Open Science Framework Repository:* https://osf.io/upka4/.
Other		
3T MRI Scanner	Siemens	Magnetom Trio or Prisma
Graduated Cylinder (at least 250 mL)	N/A	N/A
Digital Scale	N/A	N/A
Weigh Boat	Fisher Scientific	08732116
Spatula with Scoop	N/A	N/A
60 mL Syringes	Becton, Dickinson & Co	REF 309653
Male Luer to 1/8″ Barb Adapter	Cole-Parmer Instrument	SKU #: 30800-24
Female Luer to 1/8″ Barb Adapter	Cole-Parmer Instrument	SKU #: 45500-04
Three-Way Stopcock, Male Luer Lock	Cole-Parmer Instrument	SKU #: 30600-02
Tubing (for liquid delivery); ID: 1/8″, OD: 3/16″, Wall: 1/32″, Length: 50 ft	Tygon	Formulation: B-44-3
Syringe Pump	World Precision Instruments	SP200i
Portable storage containers (for liquid solutions)	N/A	N/A
RS-232 to USB adaptor	Plugable or GearMo	N/A
Serial Cable (with IBM 9 pin)	World Precision Instruments	Order code: 15623
Pacifier (or alternative device to secure tubing)	NUK	N/A

MATERIALS AND EQUIPMENT

Below, additional information is provided regarding the materials required to create recipes for the neutral and saltwater solutions for liquid delivery, along with a detailed description of the syringe pump model used to administer liquids in the study, required adaptors, custom software used to implement the study, plus notes on alternative methods and equipment.

Saltwater solution

The saltwater solution can be made up to 24 h in advance; if made ahead of time, place the solution in the refrigerator (36–38 degrees F). Once you are ready to use the solution, take it out of the refrigerator and allow it to come to room temperature (66–72 degrees F) prior to starting the experiment.

Reagent	Final concentration	Amount
Sodium Chloride	0.3 M	8.8 g
Distilled H_2O	N/A	500 mL
Total	**0.3 M**	**500 mL**

Neutral solution

The neutral solution can be made up to 24 h in advance; if made ahead of time, place the solution in the refrigerator (36–38 degrees F). Once you are ready to use the solution, take it out of the refrigerator and allow it to come to room temperature (68–72 degrees F) prior to starting the experiment.

Reagent	Final concentration	Amount
Sodium Bicarbonate ($NaCHO_3$)	0.6 mM	0.0495 g
Potassium Chloride (KCl)	6.3 mM	0.4668 g
Distilled Water (H_2O)	N/A	1,000 mL
Total	N/A	1,000 mL

Syringe pump

The liquids were dispensed using an SP200i Syringe Pump by World Precision Instruments, a microprocessor-controlled pump that allows for high metering precision. This pump model takes up to 2 syringes and only allows for infusion, although other models are more sophisticated. The syringes are stabilized using a syringe clamp, with the edge of the syringe locked into the pusher block. More details about the equipment can be found in the Instruction Manual: https://www.wpiinc.com/media/wysiwyg/pdf/SP200_IMs.pdf.

The syringe pump is connected to a USB port on a laptop computer (Windows 10; version 21H2), which requires a USB Serial adaptor (USB to RS-232). Two types of serial adaptors have worked well with this setup, including Plugable and GearMo. The main difference between these adaptors is that they contain different chips (Plugable: PL2303hx; Gearmo: FTDI). This will be connected to a Serial Port to Phone Jack adaptor (IBM 9-pin "D" connector) and critically requires a **STRAIGHT** phone cord (not a CROSSED phone cord, which can be used for daisy-chaining multiple syringe pumps). The straight phone cord will come with the adaptor. If using a Windows computer, the USB should be plugged into the correctly configured COM port. This particular setup is specific to the software programs used in this protocol, which are listed in the key resources table. This specific setup was not tested in other operating systems (e.g., Mac) or experimental task software programs (e.g., Eprime), though we believe that it should be compatible across multiple platforms and the syringe pump has been controlled by Eprime in prior behavioral experiments from the lab.

Software

MATLAB (MathWorks; Version, 2016b) and Psychtoolbox (http://psychtoolbox.org/; version 3.0.12) are used in this protocol for task administration (e.g., presentation of visual stimuli, recording of button presses), which also includes sending triggers to the syringe pumps to dispense liquid during trials in which the participants are both accurate and faster than the criterion RT. Specifically, you will need the "IOPort" function from Psychtoolbox (http://psychtoolbox.org/docs/IOPort) to open the serial port for the connected syringe pump. Importantly, extracting from the syringe the baud rate is crucial for successfully sending triggers to the pump (which can be modified in the pump), as specifying the incorrect baud rate will now allow the pump to interface with MATLAB. Critically, identifying the correct COM port for the USB connection (this can be configured manually within Windows software) is also crucial, as specifying the incorrect port will not allow the pump to work. To dispense the bolus of liquid from the task scripts, you will need to call the 'RUN' command followed by a carriage return using the "IOPort" command. After the 'RUN' command is complete, you will want to flush the data queued to be written out by the specified device (i.e., the pump). Extensive detail on how the IOPort function can be adapted for behavioral tasks can be found on the Psychtoolbox website.

Relatedly, the "CMUBox" function from Psychtoolbox (http://psychtoolbox.org/docs/CMUBox) can be used to interface the MRI-compatible response button box (fORP; Cambridge Research Systems) with MATLAB. Specifically, this function opens the COM port corresponding to the USB port connected to response button box, and allows for detection of button presses. Finally, at the end of the script, it is necessary to close the ports. Otherwise, MATLAB will not be able to run the pump in different scripts without restarting the software program. Further details on how the CMUBox function can be adapted for behavioral tasks can be found on the Psychtoolbox website.

All task scripts can be found on the project OSF, and the following task codes can be found in the main script for running the task, entitled "LiquidFeedbackfMRI.m", as well as the task for running the liquid feedback task "LF_taskfMRI.m". Notably, these codes only open the and close the computer ports connected to the equipment, as well as administer the bolus of liquid triggered by the task scripts. If the experimenter wants to change the amount of liquid dispensed, they should adjust the syringe pump manually and should reference the syringe pump's instruction manual accordingly. As the purpose of describing these task codes is to provide the experimenter with the flexibility to adapt task-triggered liquid bolus delivery, we have only described the relevant commands for interfacing the MATLAB with the syringe pump, as task scripts significantly vary between experiments; experimenters may choose to deliver liquids based on different performance criteria.

Finally, we acknowledge that the current protocol describes how the syringe pump can interface with MATLAB and Psychtoolbox. However, other programs, such as E-prime, can be used for these purposes and have been used in prior laboratory studies (Beck et al., 2010; Chiew and Braver, 2016; Crawford et al., 2020; Jimura et al., 2013; Krug and Braver, 2014; Yee et al., 2016, 2019).

Example START of task code to open the computer ports

```
% Opens the port for the syringe pump and button box
% Syringe pump
cs='BaudRate=9600 Databits=8 Stopbits=1 FlowControl=None Parity=0 SendTimeout=1 Receive-Timeout=1 InputBufferSize=64 DTR=1 RTS=1';
[pump,e] = IOPort('OpenSerialPort','COM3',cs)
% Opens the port Response button box
cs = 'BaudRate=19200 Databits=8 Stopbits=1 Parity=0 norelease=1';
handle = CMUBox('Open','pst','COM7',cs);
```

Example END of task code to close the computer ports

```
% Close the ports for syringe pump and button box
CMUBox('Close', handle);
IOPort('CloseAll');
```

Example task code to dispense the liquid bolus from syringe pump

```
% Runs the pump as is ("start"). This must end with a carriage return and line feed (CR + LF). In ASCII, they are 13 and 10, respectively
IOPort('Write',pump,uint8(['RUN' char(13) char(10)]),1);
% Flushes the data queued for writeout to device specified by pump
% handle, wait for full write completion
IOPort('Flush',pump);
```

STEP-BY-STEP METHOD DETAILS

Prepare the liquid solutions

Timing: 15 min

The objective of this step is to create the neutral and saltwater solutions used as liquid feedback in the study protocol. The neutral solution is designed to be a tasteless solution similar to artificial saliva (O'Doherty et al., 2001; Veldhuizen et al., 2007, 2011). This neutral solution was used instead of water, as water has been found to activate taste cortex (i.e., insula, operculum, post-central gyrus) and may be considered appetitive (Bartoshuk et al., 1964; Zald and Pardo, 2000). After completing this step, the experimenter will have all the necessary liquids needed for the Liquid Swallowing Practice and the scan session.

1. Prepare the Neutral Solution.
   a. Measure out 250 mL of distilled water using a graduated cylinder.
   b. Put the weigh boat on the digital scale and tare the scale.
   c. Measure out 0.4668 g of Potassium Chloride (KCl).
      i. Hold the weighed amount of KCl over your mixing container (e.g., glass beaker).
      ii. Pour the distilled water over it until all the substance has washed off the boat.
   d. Repeat this with the 0.0495 g Sodium Bicarbonate ($NaHCO_3$).
   e. If there is water left in the cylinder, add it to the mixing container.
   f. Lift the mixing container and swirl the water around until most of the substance particles are not visible in the water.

   Note: You will want to mix relatively well to ensure that all reagents have gone into the solution.

   g. Once the substances are mixed, add another 750 mL of distilled water using the graduated cylinder and mix again.

   Note: In total, you should have 1 liter of neutral solution. You can check the volume using the graduation on the mixing container.

   h. Pour the solution into a portable container (that will be brought to the scan) and seal the lid.
2. Prepare the Saltwater Solution.
   a. Measure out 250 mL of distilled water using a graduated cylinder.
   b. Put the weigh boat on the digital scale and tare the scale.
   c. Measure out 8.8 g of Sodium Chloride (NaCl).
      i. Hold the weighed amount over your mixing container (e.g., glass beaker).
      ii. Pour the distilled water over it so that all the salt is washed into the mixing container.
      iii. Pour in the remaining 250 mL of distilled water.
   d. Lift the mixing container and swirl the water around until the salt is largely dissolved within the water.

   Note: You may still see a bit of salt gathered at the bottom of the container. It is okay to see a bit of salt, but try to mix until the amount is minimized as much as possible.

   e. Once the amount of salt visible in the solution is low, pour the solution into a portable container (to be brought to the scan) and seal the lid.

Prepare the liquid delivery setup

Timing: 30 min

In this step of the protocol, you will assemble the tubing and connectors that are needed to dispense liquids to participants. Each tubing setup will be composed of two merging tubes, two refill tubes, and one long mouth tube, along with stopcocks and Luer locks to be used as connectors.

3. Cut the Tubing.
   a. From the Tygon tubing, cut two short (1–3 inches) merging tubes, two medium-length (3–5 inches) refill tubes, and one long (12–13 ft) mouth tube.

   Note: The exact length of the long tube will depend on the distance of the syringe pump to the MRI scanner. See Figures 1A and 1B for labels associated with the short and medium length tubes. The long mouth tube is not pictured, but should be long enough to connect this setup in the control room to reach the participant in the scanner bed (i.e., our setup required 12 ft of tubing, but other setups may require different length depending on the scanner setup).

4. Attach the Luer locks to the stopcocks.
   a. Screw a small Luer lock onto the long end of each stopcock. This end will be called the "top" end.
   b. With the top end facing up, screw a large Luer lock onto the right end of each stopcock.
   c. Screw another large Luer lock onto the bottom end of one stopcock. [Sub-steps under the first step].
5. Assemble the Liquid Delivery Setup.
   a. On a table, arrange the stopcock/Luer lock combination(s) into a triangle that is pointing away from you (Illustrated in Figure 1B). The stopcock with three Luer locks attached should be on top.
   b. Attach the two medium-length tubes to the right side of the two lower (or "base of the triangle") stopcocks.

   Note: These will be your refill tubes. These tubes are used to fill the syringes with liquid. This is important for refilling the empty syringes after the liquid has been dispensed to the mouth tubing to reach the pacifier. Details about how the syringes are refilled are described in step 19 "Fill the syringes with each liquid."

   c. Use the small tubing to attach the "base" stopcocks to the top stopcock.
   d. Attach the tubing to the top side of the "base" stopcocks and then to the bottom and right sides of the top stopcock.
6. Repeat steps 4 and 5 two more times to have three complete Liquid Delivery Setups (one for each of the liquids).

   Note: While the current protocol only describes three liquid setups for the juice, neutral, and saltwater solutions, this protocol is flexible such that the experimenter can create as many liquid setups for each liquid that they will use in their respective studies (1 liquid = 1 liquid delivery setup). The Liquid Delivery Setup is illustrated in Figure 1E. As there are separate syringe setups for each liquid, the experimenter only needs to swap out the syringes for administering each liquid during the task.

Practice swallowing liquids

Timing: 10 min

Since drinking liquids in a supine position (e.g., lying on the back) is not natural to many participants, it is important to have participants practice drinking liquids in this position outside of the scanner environment so they are prepared to do so when inside the bore of the scanner.

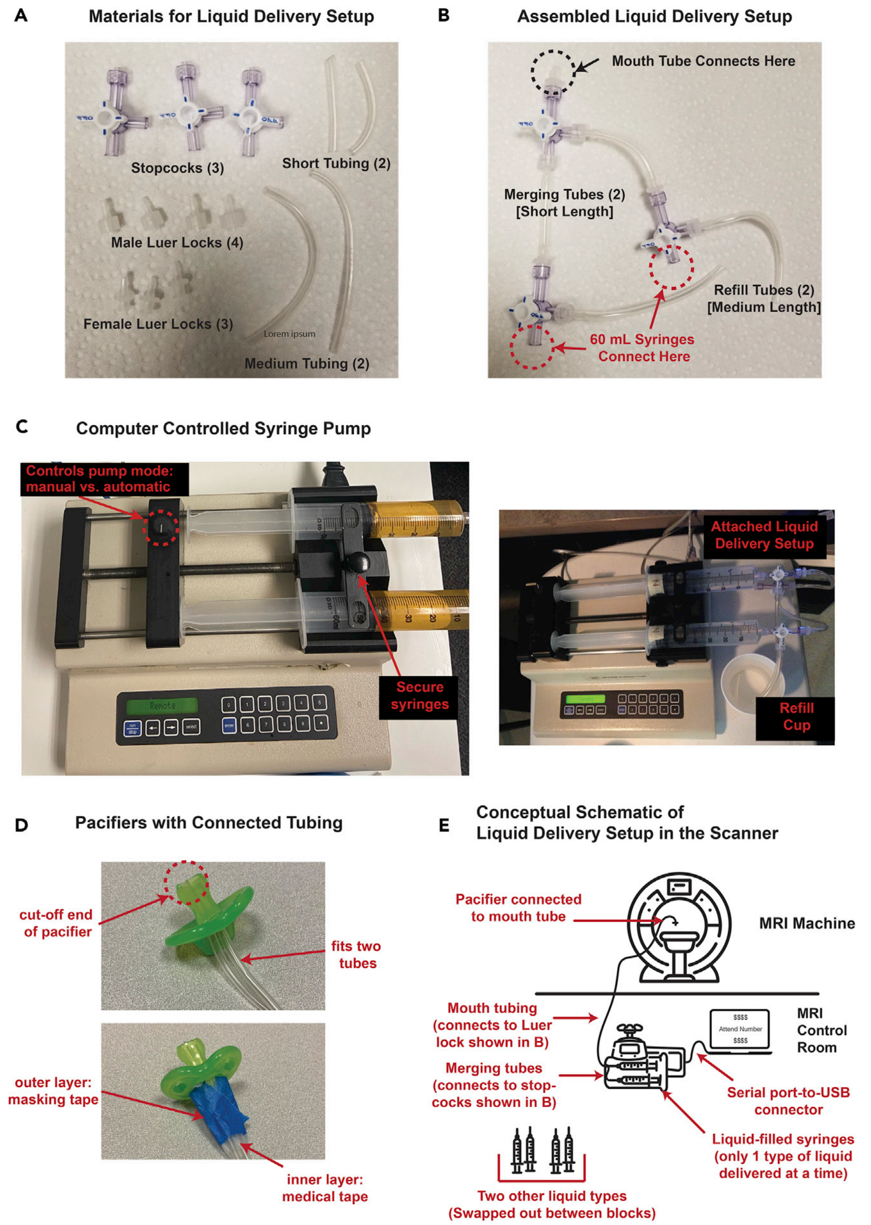

Figure 1. Preparation of liquid delivery setup

(A) Materials for Single Liquid Delivery Setup. Includes three stopcocks, four male Luer locks, three female Luer locks, two short tubes (1–3 inches) for connecting the stopcocks, and three medium-length tubes (3–5 inches) for refilling the syringes.

(B) Assembled Liquid Delivery Setup. The long mouth tubing (depends on the distance from syringe pump to MRI scanner) is connected at the end, and the two filled syringes are connected to the two stopcocks.

(C) Computer Controlled Syringe Pump Liquid Delivery Setup. The liquid is dispensed from a computer-controlled syringe pump. The syringes must be secured using the metal clasp, and the knob should be turned so that the pump mode is set to automatic (e.g., the knob should be loose).

(D) Pacifiers with Connected Tubing. The tip of the pacifier should be cut off to enable clear passage for the liquid to be dispensed. Two types of tape (e.g., masking, medical) are used to secure the tubing to the pagcifier.

(E) Conceptual Schematic of Liquid Delivery Setup in the Scanner. Once the liquid delivery setup (with the merging tubes connecting the stopcocks) is secured to the syringe pump, a long mouth tubing connects the Luer lock to the pacifier. The syringe pump is controlled by a trigger sent from the computer (here via a serial port to USB connector), which dispenses a bolus of liquid to the participant. In order to switch the liquids, the experimenter can swap out the syringes and tubing. Details about switching the liquids are provided in the protocol.

7. Fill two-60 mL syringes with the neutral solution.
8. From the Tygon tubing, cut a tube that is approximately 3 ft in length (or however long the distance is between the syringe pump and the participant).
9. Connect the end(s) of the syringes to the stopcocks in one of the assembled liquid delivery setups (See Figure 1B).
10. Secure the syringes in the syringe pump using the metal clasp on the pump (see Figure 1C).

Note: More detailed instructions for securing the syringes can be found in the manual.

11. Check that the pump is configured to dispense the liquids (e.g., priming the pump).
    a. Make sure that the knob is turned so that the pump mode is set to automatic (e.g., the knob should be loose).
    b. Check that the amount delivered is correct (e.g., to deliver 1 mL of juice per dispense, the amount should be set to 0.5 mL because there are two syringes).

Note: To change the amount of liquid administered, toggle through the settings on the pump. There will be a menu that will first ask you to set the amount of liquid to be dispensed, and after you hit enter, you can use the arrow keys on the pump to change this value and then click enter.

    c. Ensure that the liquid is flowing throughout the entirety of the tubing so that it can be dispensed to the participant by either manually pushing the liquid through the tubing with the pump or hitting the "Run" button repeatedly in the pump-controlled mode.
    d. Common issues for this step are described in troubleshooting 1.
12. Prepare the Pacifier.
    a. Cut off the tip of the pacifier to enable clear passage for the liquid dispensed through the mouth tubing.
    b. Pull the pacifier through the end of the tube so that the tube is at the edge of the opening of the pacifier (See Figure 1D).
    c. Hold the pacifier over a waste container and press "Run" on the pump repeatedly until the liquid drops into a waste container.

Note: This step is important for priming the pump.

13. Participant Practice Drinking Liquids.
    a. Have the participant lie down and hold the pacifier in their mouth by gently biting down on it. Note that too much biting or force will impede the flow of liquid.
14. Run the practice-swallowing task script.
    a. This script is called "practice_drinking_liquids.m", which can be found on OSF.
    b. Once the script has begun, you will be asked to press a key for the first practice section (single drop) and then again for the second section (five drops spaced out to simulate swallowing during the task).
    c. Make sure that you are clearly communicating with the participant when dispensing the liquids (e.g., "Now, we are going to practice swallowing five times in order to familiarize you with the pace of swallowing during the task").
15. Once the swallowing practice is finished, take the pacifier from the participant and confirm that they are comfortable with drinking the amount of liquid delivered per dispense while lying down before beginning the scan.

Optional: The liquid drinking practice can also be accomplished by manually dispensing liquid from the pump rather than using an automated script if desired.

Note: To practice swallowing liquids while lying down, it is useful to be located in a room that has a table (e.g., an exam table in a medical setting) or a reclining chair that participants can be positioned on.

Prepare liquid delivery setup in scanner

Timing: 30 min

During this step, you will transfer your liquid solutions and setups to the control room (next to the MRI scanner). These instructions describe the process of dispensing liquids to participants while they are in the bore of the scanner. Note that there is no special installation required to swap out the different liquid setups, and the syringes only need to be removed and replaced for each liquid delivery. For each liquid used in the study, one Liquid Delivery Setup was created.

16. Bring all the items for the liquid setup to the MRI control room (syringe pump, neutral syringe/stopcock setup, pacifier, and the rest of the liquid solutions and setups).
17. Setup the syringe pump and laptop computer.
    a. Place the syringe pump and scanning laptop on a small table separated from the computing equipment.

    Note: In our study, this was a pragmatic choice in order to minimize the likelihood of spilled liquids damaging the computers.

    b. Place some paper towels over the table and set the pump on it near the computers.
    c. Plug the pump into an available outlet.
18. Designate an area on the table for and prepare each liquid.
    a. You will use the same Liquid Delivery Setup for the neutral solution as in the practice session.
    b. Place your neutral setup (from the liquid drinking practice) on the table (but disconnect the syringes from the Liquid Delivery Setups to enable easier refill).
    c. In each of the other two areas, place a stopcock/Luer lock setup and two syringes.
    d. For each liquid, you will also need a filling cup and the actual liquid.
    e. Label each cup so that you never accidentally use the wrong liquid in the setup (e.g., the neutral and saltwater solutions will look identical to one another; labeling will help to ameliorate any potential mix-up).
19. Fill the syringes with each liquid
    a. Pour the liquids into their designated filling cups.
    b. Make sure that the plungers are pushed all the way through the barrel of the syringes such that the seal is closed to ensure no air bubbles.
    c. Place the syringes in the cups, and pull back the plunger to fill each syringe to 60 mL (i.e., the max volume) using the cups.
    d. Next, attach the syringes to the stopcocks/Luer lock setups similar to during the swallowing practice.
    e. Refill the neutral solution syringes with more solution before reattaching them to the stopcock/Luer lock setup since the levels will have been depleted during the liquid drinking practice.
20. Prepare the Mouth Tubing to Connect to the Liquid Delivery Setup.
    a. For each liquid, take out a length of fMRI-cut tubing. Note that there will one mouth tubing for each Liquid Delivery Setup (e.g., for 3 liquids, you will need 3 mouth tubes). The referenced study uses 25 ft long tubing, but this may be adjusted depending on the specific environment (e.g., you may opt to have a longer or shorter mouth tubing, depending on distance between the control room and the scanner).

b. Using the masking tape, create labels on each tube near each end of the mouth tubing. The label should be near the ends of the tubing but not so close that it will interfere with the tube being inserted into the pacifier.
c. On the labels, write the first letter of the corresponding liquid (e.g., "J" for "juice"). This is not strictly necessary but useful to keep all tubing separate during scanning.

21. Move the mouth tubing to the scanner room.
    a. Take your three lengths of fMRI tubing (i.e., one for each liquid type) and tape one end of each side-by-side on the table with the pump. Make sure that the labels are still visible.
    b. Once the ends are taped down, secure them further to prevent them from slipping/falling while the participant is being situated in the scanner.

Note: You can place a heavy object on the taped tubes to preven them from moving.

c. Wrap the free ends of the tubes together with some paper towels.
d. Using the opening between the control room and the scanner, slide the tubing through.

Note: Before (and while) doing this, you may need to untangle the tubes a bit.

22. Attach the mouth tubing to the pacifier used for liquid delivery.
    a. Take note of the first two liquids that will be dispensed.
    b. Insert the tubes that correspond to the first two liquids.

Note: The pacifier has space for two tubes, and these tubes will need to be changed out with the final liquid later. Alternatively, you can set up multiple pacifiers and have the participant swap out the pacifier when switching to a liquid that requires a different mouth tubing.

c. Take the tape and pacifier and enter the scanner room, also, pick up the paper towels and tubing.
d. Pull the tubes through the opening so that they can reach back to where the participant will be in the scanner.
e. If the tubes are tangled, you may need to pause here to untangle the tubes more. Importantly, while dealing with the tubes, make sure that the exposed ends do not touch the ground.
f. Insert the two tube ends that correspond to the first two liquids (e.g., Juice, Saltwater) into the pacifier.
g. Carefully tape the tubing to the pacifier.
    i. Wrap a clear medical-grade tape around the tubes and the pacifier.
    ii. Use the masking tape to provide an additional seal around the pacifier by wrapping it around the entirety of the pacifier.

Note: The current setup describes two kinds of tape to fasten the tubing to the pacifier for the scanner setup. The medical-grade tape is more pliable and helps to keep the tubing and pacifier connected. The masking tape is helpful for keeping the tubing secured.

23. Give the pacifier with mouth tubing to the participant.
    a. Wrap the pacifier in a paper towel and hang it and the free tube over the scanning table so that they are hanging freely and not touching anything.
    b. Use tape to secure tubes to the table as you situate the participant in the scanner.
    c. Once the participant is in the bore of the scanner, hand them the pacifier.
    d. Depending on the scanning protocol, you can instruct participants to keep the pacifier out of their mouths while you collect structural (or other) scans.
24. Once you are ready to begin the scans with the liquids, instruct the participant to place the pacifier in their mouth.
25. Fill the mouth tubing with liquid from the syringe pump.

a. Once the pacifier is connected to the mouth tubing, and the mouth tubing is connected to the liquid delivery setup, fill the mouth tubing with liquid from the syringe pump.

Note: You will need two people for this step (one person to push the liquids through the tubing, one person inside the scanner to monitor the liquid flowing through the mouth tubing).

b. Make sure the "OFF" knobs of the stopcocks are pointed downward to block the refill tubes.

Note: This will ensure that any liquid pushed through the syringe will go directly to the mouth tubing.

c. Make sure the pump mode is set to "manual" (the pump knob should be rigid).
d. Push the ends of the syringe pump to dispense the liquid through the mouth tubing.

Note: The other experimenter should be in the scanner room monitoring the liquid flowing through the tubing, and will tell the experimenter in the control room when to stop dispensing liquid (i.e., when the level reaches the pacifier and the participant can taste the liquid).

e. Set the pump mode to "automatic" (knob should be loose).

26. Confirm that the syringe pump dispenses a bolus of liquid prior to the start of each run.
    a. You will press the "RUN" button on the syringe pump to prime the pump (i.e., dispense a bolus of liquid) to test that the pump dispenses properly.

Note: You may need to press this a few times until the participant tastes the liquid. Once the participant tastes the liquid, you can set up the task scripts and scans.

b. Ask the participant to confirm that they received the liquid in their mouth prior to beginning each run.

Note: The participant should taste the liquid immediately after the bolus of liquid is dispensed triggered by the pump. Participants do not need to suck on the pacifier in order for the liquid to be delivered. Because the amount delivered is minimal (1 mL is about 1 drop), tasting the liquid should not elicit any head movement (you should have instructed the participant to keep their head still during the scan).

Note: Prior to starting the study, it will be critical to determine that there is an opening between the control room and the scanner through which you can run the tubing for the study.

Cleaning the materials after the scan

Timing: 1 h

After the scan has finished, you can collect the supplies and clean them for future use.

27. Take the liquid setups apart.

Note: If you used tubing that was autoclaved twice, you could throw out the tubes. Otherwise, you can save them for further use after autoclaving, if desired.

28. Clean the Materials for Liquid Delivery Setups.
    a. Rinse off the stopcocks and Luer locks.
    b. Remove the plungers from the syringes so that you can rinse out both parts.
    c. After rinsing, all connectors and syringes will need to be cleaned with hot water and bleach.

29. Clean the Tubing.
    a. To clean the tubes, run tap water through them. F ill a syringe with air and attach a small Luer lock to it so you can attach it to the tubing.
    b. Use the syringe to pump air through the tubes until most of the liquid is gone.
    c. After rinsing the tubes, prepare them for the autoclave.

Note: You will want to and autoclave according to the specifications on the machine if you plan to reuse them in the future.

Note: We recommend using new tubing for the long tubes connecting the pump to the MRI to minimize the possibility of kinks in the tubing (the tubes may become sticky after being autoclaved).

EXPECTED OUTCOMES

At the conclusion of this protocol, you should have a working setup to deliver liquid incentives to participants while they complete tasks in the MRI scanner. More specifically, for this particular design, you will have three identical liquid setups (i.e., one for each liquid incentive—juice, neutral solution, saltwater). Each of these liquid setups will be secured in a syringe pump, one at a time (i.e., manipulated in a block-wise fashion in the task), to deliver 1 mL of liquid incentive to participants on each trial in the liquid feedback task when they are both fast enough and accurate (see Yee et al., 2021 for more details). In the current setup and task, the participants can expect to drink up to 60 mL (2 oz) of each liquid during one 2.5-h session, assuming they answered correctly and fast enough on every trial, which is seldom the case. To enable the successful delivery of liquid incentives, the syringe pump will be connected to the computer you're using to administer the task using a serial port connector with custom code that will send triggers to the pump when participants are to be rewarded. As such, the pump will dispense 1 mL of liquid through the attached tubing and pacifier each time a trigger code is sent to the pump, which will result in direct liquid delivery into the participant's mouth while they are in the bore of the scanner completing the task.

LIMITATIONS

As described in the materials and equipment, this protocol is specific to how liquids were administered to human participants based on a recent fMRI study from our lab (Yee et al., 2021). Although certain details of these protocols are specific to our MRI scanner setup, most of these procedures can be adapted to other MRI or behavioral experimental setups in the laboratory. Finally, we chose the concentrations for neutral and saltwater solutions based on prior subjective ratings of these liquids (Yee et al., 2016), and researchers may choose to utilize different concentrations based on experimental needs and/or subjective taste preferences.

TROUBLESHOOTING

Below we describe a few common experienced problems that might arise from running the protocol and provide possible solutions.

Problem 1

Liquid is not being delivered to the participant.

Potential solution

There are many possible reasons why the liquid may not be dispensing properly, relevant to protocol step 26 in the Prepare Liquid Delivery Setup in the Scanner section. Below, we have organized the potential troubleshooting avenues to pursue when you encounter this issue.

Syringe pump:

- Ensure that the pump is plugged in and turned on.
- Check the controls of the pump.
  - The WPI syringe pumps used in this protocol have two control modes: manual and automatic.
  - If the knob on the syringe pump is rigid (see Figure 1C), the pump is in manual mode and will not dispense when triggers are transmitted to it.
  - To change the pump mode from manual to automatic, simply turn the knob until it can be freely moved; the pump will now respond to triggers sent to it from the scanning computer.

Software:

- Check to make sure that the computer and/or task configurations are communicating properly with the pump.
  - As detailed in the materials and equipment section, you may need to add additional code to your task scripts in order to open and close the serial port to the syringe pump in order to dispense the liquid.
  - See task code on OSF for further details.
- Ensure the proper device drivers are installed on the scanning computer.

Note: This is important for the USB to RS-232 adapters that are being used to communicate with the port(s) used to transmit the triggers. The relevant drivers associated are also detailed under the "syringe pump" section of the "materials and equipment."

Tubing:

- Examine the length of the tubing to make sure that there are no kinks that prevent the liquid from being dispensed.
- Ensure that the "OFF" knobs on the stopcocks are pointed in the correct directions so as to not impede the flow of liquid through the tubing.

Note: If the liquid is not properly dispensing, it would be helpful to check that these knobs are aligned correctly.

- Check that the liquid can be properly dispensed to the participant (e.g., you can press the RUN button to dispense the liquid). If the liquid is not dispensing, it is likely because the knob (see Figure 1C) on the syringe pump is not properly loosened. Loosening the knob should allow you to dispense the liquid.

Problem 2

Tubing falls out of the participant's mouth prior to/during the scan.

Potential solution

In our protocol, we used a pacifier to help to fasten the tubing to a device that can sit comfortably in the participant's mouth during the scan. Depending on the specifics of your protocol, you may wish to go with a different kind of setup (e.g., a cage that dispenses liquid drops).

If you do end up using a pacifier, you should be aware that there are some pitfalls. One of which is that the tubing can sometimes come loose from the pacifier during the scan. As discussed above in the protocol step 22, two kinds of tape were used to secure the tubes in the pacifier (medical tape) and shield against moisture/leakage (masking tape) to mitigate this problem. Nevertheless, you may need to go into the scanning room to adjust the pacifier if it comes loose. If this happens, we recommend bringing a paper towel to dry off the tubing and pacifier and then reapplying the two kinds of tape mentioned above. If you have already gone through one of the types of liquids, you can also use this time to switch out the tubing in the pacifier to prepare for future runs.

You should also be cognizant of the type of head coil that is being used and how this interacts with the tubing-pacifier setup. Some head coils might obscure direct access to the participant's mouth. If this is the case, you will likely want to lift the pacifier & tubing above the coil and thread it through rather than trying to push it under the coil. Any major bending and/or pulling on the pacifier-tubing setup can cause the tubing to come loose. We recommend piloting this setup prior to running any participants in the scanner for data collection purposes.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Todd Braver (tbraver@wustl.edu). All technical questions regarding the implementation of the protocol can be directed to Debbie Yee (debbie_yee@brown.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The datasets and code generated during this study (Yee et al., 2021) are available on the Open Science Framework Repository: https://osf.io/upka4/.

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AUTHOR CONTRIBUTIONS

D.Y.: Conceptualization, Methodology, Investigation, Writing-Original Draft, Visualization, Funding Acquisition. J.C.: Methodology, Investigation, Writing-Original Draft. T.B.: Conceptualization, Supervision, Writing-Review & Editing, Funding Acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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