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RESEARCH ARTICLE

Stimulus temperature modulates the psychophysical dimensions of salt taste

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Abstract

Temperature is a fundamental yet understudied parameter of gustatory experience. In this study, we designed a set of experiments that examined how stimulus temperature affects the motivational and sensory-discriminative properties of sodium (NaCl) and nonsodium (KCl) salts. In brief-access tests, water-deprived mice consistently preferred cool (14°C) over warm (36°C) stimuli regardless of salt content. Progressive ratio tests confirmed that cool stimuli possess greater appetitive efficacy, suggesting that, under our experimental conditions, water deprivation may confound temperature preferences in brief-access procedures. In contrast, operant conditioning tasks revealed robust temperature effects on sensory-discriminative function. In a two-response taste detection task in which mice were trained to distinguish each salt from water, enhanced sensitivity to both NaCl and KCl was observed at 36°C compared with 14°C, with detection thresholds significantly lower at warm temperatures. Critically, in discrimination tasks where mice were required to distinguish between NaCl and KCl with stimulus intensity controlled by anchoring concentrations at their respective detection thresholds, mice showed significantly impaired discrimination performance at 36°C compared with 14°C, indicating that the qualitative signatures of the two salts became perceptually more similar at the warmer temperatures. These findings demonstrate that temperature profoundly modulates the sensory-discriminative properties of salts: although warm temperatures enhance detection sensitivity, they simultaneously reduce qualitative discriminability between sodium and nonsodium salts. Overall, these results have implications for the operational principles of taste function and highlight the promising possibility that temperature manipulation could serve as an effective strategy for dietary sodium reduction.

NEW & NOTEWORTHY Despite being a fundamental parameter of gustatory experience, how temperature shapes taste perception remains poorly understood. Using a multifaceted behavioral approach in mice, we found that water deprivation confounds temperature-driven motivational responses. In contrast, warm temperatures both lowered detection thresholds for NaCl and KCl and impaired discrimination between the two salts, revealing that temperature modulates the perceptual similarity between sodium and nonsodium salts with implications for taste neuroscience and sodium reduction strategies.

psychophysics; salts; taste; thermo-gustation; thermo-sensitivity

INTRODUCTION

One of the central aims of gustatory science is to understand the neural mechanisms subserving taste perception. Such efforts must start with a clear delineation of the relationship between the properties of the oral stimulus and perceptual outcome. To date, the vast majority of chemosensory research focused on gustation involves taste stimuli presented at room temperature. However, in the natural environment, animals are exposed to chemical stimuli at a variety of temperatures; this is especially true in humans, for whom refrigeration and cooking come into play. Accordingly, temperature represents a basic physical parameter of taste stimuli that can influence gustatory processing via both direct effects on the peripheral sensory apparatus and central integration of trigeminal and taste signals. Every stimulus introduced into the mouth possesses a temperature (whether as an absolute value or relative to the temperature of the oral cavity), making this physical attribute an unavoidable and relevant feature of the gustatory experience. Thus, temperature must be considered in any analysis of gustatory function, much like concentration and molecular structure.

Neurophysiological studies in both anesthetized preparations and, more recently, awake behaving rodents have demonstrated that fluid temperature strongly modulates neural activity throughout the gustatory pathway, from the cranial nerves to the gustatory cortex (1–9). Despite this neurophysiological evidence for temperature-taste interactions, behavioral studies of taste perception in laboratory animals have been conducted predominantly at room temperature, with comparably little effort directed toward elucidating the role of oral temperature sensation in food and fluid intake (10). To assess how experimental manipulations affect taste-guided behavior, it is important to recognize that taste

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function can be heuristically divided into three domains (11, 12): *1*) sensory-discriminative function, which involves processes that identify the stimulus quality (what is it?) and intensity (how strong is it?) of the stimulus, *2*) motivational/affective function, which reflects the hedonic value and reinforcing efficacy of the stimulus that promotes or discourages ingestion (how good or bad is it?), and *3*) physiological function, the so-called cephalic phase reflexes (e.g., Ref. 13), that facilitate the optimal digestion and assimilation of nutrients. The few behavioral studies that have examined stimulus temperature have done so exclusively within the motivational/affective domain, using brief-access and two-bottle preference tests to assess motivated responsiveness in water-deprived rodents, and have shown that cooling sucrose from 20° to 10°C decreases licking (14, 15). However, it is important to note that the domains of taste function are partially dissociable. For example, two stimuli like a bitter and a sour taste may be equally avoided (same motivational value) yet remain readily distinguishable based on their qualitative characteristics (different sensory-discriminative properties). This dissociation underscores the necessity of using complementary behavioral approaches to comprehensively assess how variables like temperature modulate taste perception.

On this basis, we reasoned that stimulus temperature might not act uniformly across taste function and may exert separable—and potentially opposing—effects on its motivational and sensory-discriminative domains. This possibility is especially compelling, and largely untested, for salt taste. Rodent studies of temperature effects on salt taste are absent, and the human evidence is limited and contradictory: warming from 25° to 35°C increased perceived taste intensity of NaCl in one study (16) but had no effect in another spanning 20° to 36°C (17). More fundamentally, no study has asked whether temperature alters the qualitative character of salt taste—whether warming changes not merely how strong a salt tastes but what it tastes like.

We therefore hypothesized that temperature shifts not only the intensity but also the perceived quality of salts, such that warming alters the qualitative similarity between a sodium salt (NaCl) and a nonsodium salt (KCl)—a shift that would carry implications for the neural processing of salt taste and for sodium-reduction strategies in foods served at different temperatures. To test these hypotheses, we took a multifaceted behavioral approach to systematically examine how temperature modulates salt taste perception across the motivational and sensory-discriminative domains. We tested adult mice with NaCl and KCl solutions presented at nonnociceptive cool (14°C) and warm (36°C) temperatures using complementary behavioral paradigms, each designed to isolate specific aspects of taste function. Brief-access and progressive ratio tests assessed the motivational aspects of the stimulus, whereas operant detection and discrimination tasks assessed sensory-discriminative aspects. We found that in water-deprived mice, cool drives appetitive responding more than warm stimuli regardless of salt identity or concentration, rendering brief-access procedures poorly suited for assessing temperature effects on salt palatability under these conditions. In contrast, temperature profoundly influenced sensory-discriminative processing: warm temperatures lowered detection thresholds for both salts (enhanced sensitivity), yet simultaneously impaired discrimination between NaCl and KCl when stimulus intensity was controlled (reduced qualitative discriminability). Our findings reveal that temperature modulates the perceptual similarity between sodium and nonsodium salts, with potential implications for the operational principles of taste function and salt reduction strategies in human nutrition.

METHODS

Subjects

Adult female and male wild-type C57BL/6J (B6) mice (n = 114; Jackson Laboratory, Bar Harbor, ME) aged 22–25 wk at the start of behavioral training served as subjects. All mice were experimentally naïve at the start of the experiments. The mice were housed in groups of three and kept in a colony room where the temperature, humidity, and lighting (12:12-h light-dark) were automatically controlled. The mice were given free access to laboratory food (LabDiet 5001; PMI Nutrition International, Brentwood, MO) and water for 1 wk after arrival to acclimate to the laboratory environment. After 7 days of habituation, mice were placed on a water-restriction schedule in which they received a total amount of 1.5 mL of water each day that they obtained during testing with the remaining amount given in separate, individual cages if the total amount was not reached during the experimental session. The body weights of the mice were monitored daily to ensure that they remained at or above 80% of their pre-restriction weight. If a mouse dropped below 80% of their free-drinking weight, it received a daily total of 2 mL of water until their weight was restored. Training and testing took place during the light-on cycle, Monday through Saturday. On Sunday, the mice were temporarily (~60 min) separated into individual cages to receive their 1.5 mL of water. Out of the 114 mice, 18 were removed due to not progressing through the training stages of the different experiments (see *Experimental Design and Data Analysis*). As a consequence, the number of experimental subjects that were included in the analyses is 96 (49 male and 47 female). Given that the number of subjects per experimental procedure did not allow us to treat sex as an independent variable, data from male and female mice were collapsed across analyses; future experiments will be designed to systematically evaluate potential sex differences in temperature-dependent salt taste perception. Sample sizes were not determined by a formal a priori power analysis but were chosen to be consistent with previous rodent taste behavioral studies using brief-access, progressive ratio, and operant detection and discrimination paradigms, which have reliably detected effects of comparable magnitude with similar group sizes. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Florida State University (IACUC) under the protocol "PROTO20210000."

Taste and Thermal Stimuli

All taste solutions were prepared fresh daily with nanopure water (Barnstead/Thermolyne Nanopure laboratory water system) and reagent-grade chemicals (Fisher Scientific). During the brief-access test experiments, three concentrations of NaCl (0.15, 0.6, and 0.9 M) and KCl (0.2, 0.6, and

0.8 M) spanning mild to aversive intensities were selected based on prior concentration-response characterizations in rodents (18, 19). Test stimuli during the operantly conditioned taste detection phase of the study consisted of 10 concentrations of NaCl (0.00075, 0.001, 0.003, 0.006, 0.02, 0.04, 0.07, 0.15, 0.3, and 0.6 M) and KCl (0.001, 0.002, 0.004, 0.01, 0.02, 0.04, 0.08, 0.2, 0.4, and 0.8 M); these concentration ranges were selected to span subthreshold to suprathreshold levels based on previous psychometric threshold studies of NaCl and KCl in rodents (20). Finally, during the operantly conditioned taste discrimination phase of the study, test stimuli consisted of three concentrations for each stimulus temperature of NaCl (14°C: 0.04, 0.07, and 0.11 M; 36°C: 0.02, 0.03, and 0.05 M) and KCl (14°C: 0.33, 0.53, and 0.84 M; 36°C: 0.17, 0.26, and 0.42 M). These concentrations were anchored to the mean detection thresholds (EC_{50}) obtained from the operantly conditioned taste detection experiments (EC_{50} + 0.25, 0.45, and 0.65 log units), ensuring that NaCl and KCl occupied overlapping positions on their respective psychometric functions and rendering stimulus intensity an unreliable cue for discrimination. Water and salt solutions were presented at 14° and 36°C. These temperatures were selected for several reasons: *1*) both fall outside the range of overtly noxious thermal stimuli (21, 22), *2*) they are sufficiently separated on the temperature scale to allow initial investigation of the role of stimulus temperature in salt taste perception before examining finer thermal differences, *3*) they afford compatibility with prior behavioral studies of temperature effects on taste in rodents (15), and *4*) they are on the cooling and warming range as compared with the reported resting oral temperature in mice (3).

Temperature-Controlled System

To achieve precise temperature control, the licking spouts were directly embedded within custom-built Peltier-block temperature controllers, as already in part described in Ref. (1). In brief, fluid was delivered via gravity through computer-controlled 12-V solenoid valves, calibrated daily to deliver 4 μL from both the central licking spouts (used in all experiments) and the side ports for reinforcements (used in operant conditioning experiments). By embedding the licking spouts within the Peltier devices, we could heat or cool the spouts to precise temperatures ranging from 0° to 50°C. This was accomplished by altering the polarity and magnitude of the DC current supplied by a central amplifier, which adjusted the temperature of the aluminum block housing the licking spout. The Peltier devices maintained the set temperature with an accuracy of ±0.1°C, ensuring that the fluid delivered was thermally consistent across presentations. To precisely calibrate and monitor the temperature of the drinking solution, a thermocouple probe was placed inside the Peltier in direct contact with the licking spout near its exit point; this sensor confirmed that thermal variability at the spout tip remained within the 0.1°C tolerance. In some cases, a second thermocouple (ALA Scientific Instruments) was used to independently verify and calibrate the Peltier temperature readings. This design provided the necessary precision to control both the temperature and volume of fluids dispensed throughout all experiments.

Behavioral Apparatuses

Davis Rig.

Brief-access tests and progressive ratio experiments were conducted in a modified Davis Rig behavioral testing device (Med Associates Inc.) consisting of a polycarbonate cage with a wire mesh floor and a centrally located opening that is occluded by a computer-controlled shutter (Bpod, Sanworks). A computer-controlled sliding table with two drinking spouts, each embedded in a Peltier temperature controller, was positioned on the other side of the shutter. Each spout contained smaller tubes inside connected to separate lines that dispensed water or the target taste/s at a specific temperature (14° or 36°C). This minimized both the possibility of contamination between stimuli and any differences in flow rate. This device allowed each animal brief access (on the order of seconds) to a single drinking spout through the centrally positioned slot in the front wall of the cage when the shutter was open. Lick contact was measured by a specially designed high-frequency AC electrical circuit coupled to a contact lickometer that minimized electrical stimulation of the tongue, and lick responses were recorded by Bpod (Sanworks) software for later analysis. Background white noise was presented for the entire duration of experimental sessions to minimize the possibility of extraneous auditory cues.

Operant tasks.

Operant conditioning experiments were conducted inside a modified behavioral box (Sanworks) containing three behavioral port chambers. The central port was hollow to accommodate, when available, a central steel spout embedded in a Peltier. As described earlier for the Davis Rig, the spout contained smaller tubes connected to separate lines that dispensed water or target tastes at specific temperatures (14° or 36°C), minimizing contamination and flow rate differences. Water and target tastes were alternated between the inner lines of the central spout each day to ensure that stimulus-side port assignments and performance were based solely on chemical stimulus rather than extraneous cues. Lick detection for the central spout was performed as described earlier for the Davis Rig. The two side ports were used as response manipulanda and for water reinforcer delivery. Side port entries and reward contingencies were detected via nose-poke and monitored by infrared beams. As with the Davis Rig, background white noise was presented throughout experimental sessions to minimize auditory cues from solenoid valves.

Experimental Design and Data Analysis

Brief-access test.

In this task, water-deprived mice were allowed to consume a stimulus freely for a very short period of time (here, 10 s) in randomized trials across concentrations and temperature. Nineteen (8 females and 11 males) mice were used for this task. The temporal design of this experimental protocol was as follows: on the first day of training, water-deprived mice were individually placed on the Davis Rig and allowed to lick water ad libitum from a stationary spout with water at room temperature on all lines and while the shutter remained open for a period of 2 min. After these 2 min, and the next

day, a shutter-controlled protocol was run to habituate the mice to the shutter opening and closing, with all lines still containing water at room temperature. The testing began on *day 3* and continued until *day 8*, with three sessions dedicated to each salt (see Fig. 1*A* for timeline figure). Across the testing sessions, salt presentation order was counterbalanced across mice: half started with NaCl, whereas the other half with KCl. To control for potential side biases or nongustatory cues, the Peltier temperature assignments were switched daily. Each testing session consisted of 32 trials with 4 presentations of each concentration-temperature pair presented in pseudorandom order. Thus, salts were offered at both temperatures in each testing session. Each trial began with the shutter opening, giving the mouse 10 s to initiate a lick. If licking was initiated, the timer reset, and the stimulus was available for sampling for an additional 10 s, after which the shutter closed, and the intertrial interval began. Trials in which the mouse failed to initiate a lick were excluded from analysis. Following the stimulus presentation, there was a 3.5-s delay before the shutter opened again to present water (as a rinse) for 2 s. Between each trial, there was a 23-s intertrial interval. This design allowed each animal to compare all salt concentrations at both temperatures within a single session, providing a direct within-session assessment of the effects of temperature on hedonic responses. Brief-access test data were analyzed using two-way repeated-measures ANOVAs with temperature and concentration as within-subjects factors. Two sets of analyses were performed: one on raw lick counts and another on normalized lick counts (licks to each salt concentration divided by licks to water at the corresponding temperature) to control for baseline temperature preferences. Following significant main effects or interactions, post hoc pairwise comparisons were conducted using estimated marginal means with Bonferroni correction to assess temperature effects within each concentration and concentration effects within each temperature. NaCl and KCl were analyzed separately.

Progressive ratio test.

In this task, water-deprived mice were trained to lick a dry spout to receive a drop (4 μL) of water reinforcement at a specific temperature. The initial requirement for the reinforcer is low, but the requirement increases progressively during the session until the mice reach a breakpoint and stop responding any further (23). Twelve mice (6 females and 6 males) were used for this task. The temporal design of this experimental protocol was as follows: on the first day of training, water-deprived mice were individually placed on the Davis Rig and allowed to drink water ad libitum from a stationary licking spout with water at room temperature for a period of 2 min. After these 2 min and the next day, a shutter-controlled protocol was run to habituate the mice to the shutter opening and closing. The testing began on *day 3* and continued on *day 4*, with two daily sessions dedicated to each stimulus temperature (water at 14° or 36°C; see Fig. 3*A* for timeline figure). Stimulus temperature order was counterbalanced between mice: half started with water at 14°C, whereas the other half started with water at 36°C. During testing sessions, mice worked for water reinforcement on a linear progressive ratio schedule (PR3), in which the number of dry-spout licks required to earn a single 4 μL water droplet increased by three licks on each successive trial (3, 6, 9, 12, etc.). Each trial began with the shutter opening to provide access to the dry spout. A trial was considered complete when the mouse met the response requirement, at which point a water droplet was delivered, and a 7-s intertrial interval began before the next trial. A trial was considered incomplete if the mouse failed to complete the required number of licks within 60 s of initiating licking. The session ended when a mouse failed to complete three consecutive trials, establishing the breakpoint as the highest ratio successfully completed. Statistical analysis of progressive ratio data was conducted using the Wilcoxon rank sum nonparametric to compare breaking points and completed trials between temperatures, with each mouse serving as its own control across the two temperature conditions. Cohen's *d* was calculated to assess effect size for paired samples. The normality assumption was verified by applying Shapiro–Wilk tests to the difference scores (14°C–36°C) for each dependent variable.

Operantly conditioned salt taste detection.

To assess how temperature affects salt detection sensitivity, we used a two-response operant taste detection task in which mice were trained to distinguish a salt stimulus (NaCl or KCl) from water at a specific temperature. This task measures the minimum concentration at which mice can reliably detect the presence of the salt independent of the hedonic properties of the stimulus. Forty-six mice were assigned to one of the four conditions based on target salt (NaCl or KCl) and stimulus temperature (14° or 36°C): NaCl at 14°C ($n = 11$), NaCl at 36°C ($n = 9$), KCl at 14°C ($n = 16$), and KCl at 36°C ($n = 10$). Within each condition, the side port associated with the target salt was counterbalanced between males and females.

Training and testing procedures followed established protocols for rodent taste psychophysics using two-response operant detection tasks (20). The temporal design of this experimental protocol proceeded through four sequential training phases followed by the testing phase. In brief, it was as follows. In the first training phase, water-deprived mice learned to nose-poke the central port to receive water rewards. The side ports were inaccessible during this phase. A brief auditory cue (500 ms, 75 dB, 6 kHz) signaled the availability of the sampling spout, through which mice received a 4-μL drop of room temperature water upon central port entry. Mice advanced to the next phase after successfully completing more than 60 consecutive trials (typically 2–3 days). In the second training phase, mice learned to associate each side port with a specific stimulus identity. The central spout pseudorandomly delivered 4 μL drops of either water or the target salt (NaCl or KCl) at the assigned temperature (14°C or 36°C). Mice had 5 s to initiate each trial by entering the central port and make a choice by entering one of the two side ports. Correct choices resulted in delivery of a 4-μL room temperature water reinforcement from the chosen side port. For incorrect choices, mice were required to nose-poke the opposite (correct) side port to receive the water reward before the next trial began. This correction procedure ensured mice learned the stimulus-port associations. Mice progressed to the third phase after completing at least 80 trials with performance exceeding

60% (typically 1–2 days). In the third training phase, the correction procedure was removed: only correct initial choices were reinforced. Beginning in this training phase and continuing into the testing phase, incorrect responses resulted in a 2-s time-out period before the next trial began. To prevent the development of side biases, if a mouse exhibited >10% difference in correct performance between the two ports, additional trials were presented for the port with lower performance. Mice continued in this bias-correction protocol until they no longer displayed a bias (<10% difference between ports) and successfully initiated at least 150 trials (typically 1–5 days). In the fourth and final phase of training, water and target salt (at the highest suprathreshold concentration) were presented pseudorandomly without bias correction. Mice advanced to the testing phase after successfully initiating more than 150 trials with active performance (trials that were initiated and with a response) exceeding 90% for two consecutive days.

During the testing phase, sessions alternated between control and detection sessions (1 session per day). On control days (e.g., Mondays, Wednesdays, and Fridays), mice discriminated water from a previously mastered salt concentration to maintain stable performance. On detection days (e.g., Tuesdays, Thursdays, and Saturdays), progressively lower salt concentrations were tested. Mice began testing with the highest concentration of their assigned target salt (NaCl: 0.6 M; KCl: 0.8 M) as the control. On detection days, the concentration was reduced in decreasing order (NaCl: 0.00075–0.6 M; KCl: 0.001–0.8 M) in steps of ~0.32 $\log_{10}$ units, following established protocols (20). If a mouse performed more than 80% on a detection day, that concentration became the new control concentration for subsequent control sessions. Once performance on a detection day fell below 80%, the lowest concentration that resulted in ≥80% performance was used as a control for the remainder of testing. This adaptive procedure ensured that stimulus control was maintained throughout testing while accounting for individual differences in sensitivity and performance.

After testing all 10 concentrations, the mice completed two water control sessions to verify that the performance was not driven by extraneous signals. During water control sessions, both lines within the central spout contained water, with one line designated as the "target" stimulus. The testing protocol was run normally to confirm that the performance returned to ~50% (chance level). This was repeated for a second day with the opposite line designated as the target to ensure that the mice were not learning daily alternation patterns. Successful return to chance performance confirmed that mice relied on gustatory cues rather than nongustatory factors to guide their choices.

Individual psychometric functions were generated for each mouse by fitting a logistic equation to the concentration-response data using nonlinear regression (24):

$$f(x) = \frac{a - 50}{(1 + 10^{(x-c)b})} + 50,$$

where a is the maximum asymptote of performance, b is the slope of the psychometric function, c is the $\log_{10}$ stimulus concentration corresponding to half of the maximum asymptote of performance (i.e., EC_{50}, the detection threshold), and x is the stimulus concentration in $\log_{10}$ units.

Statistical comparisons of detection thresholds (parameter c) and psychometric slopes (parameter b) between temperatures were conducted using independent samples tests, as different groups of mice were trained at each temperature. For each parameter, normality was assessed using Shapiro–Wilk tests and homogeneity of variance was evaluated using F tests. When both normality and equal variance assumptions were met, Student's independent samples t tests were used. When variances were unequal, but data were normal, Welch's t tests were applied. When normality was violated, nonparametric Mann–Whitney U tests were used. Separate analyses were conducted for NaCl and KCl detection data. Effect sizes were reported as Cohen's d for parametric tests and rank-biserial correlation for nonparametric tests.

Operantly conditioned salt taste discrimination.

To assess how temperature affects salt quality discrimination, we used a two-response operant discrimination task in which mice learned to distinguish between NaCl and KCl at a specific temperature. Unlike the detection task, which, in the context of our work, measures sensitivity to stimulus intensity (can the animal detect the presence of a salt?), the discrimination task measures the ability to distinguish between qualitatively different stimuli (can the animal distinguish NaCl from KCl?). To isolate quality-based discrimination from potential intensity confounds introduced by temperature-dependent threshold shifts, we used salt concentrations anchored to the detection thresholds obtained in the previous experiments. We conducted two complementary discrimination experiments: *Experiment 1* used a within-subjects design in which each mouse was tested at both temperatures across multiple concentration pairs, whereas *Experiment 2* used a between-subjects design with separate groups trained and tested exclusively at either 14°C or 36°C. The methods for each experiment are described separately in *Experiment 1: within-subjects design across multiple concentrations* and *Experiment 2: between-subjects design with temperature exposure from initial training*.

Experiment 1: within-subjects design across multiple concentrations. Fourteen mice were trained to discriminate NaCl from KCl at three concentration pairs matched for perceptual intensity at each temperature. Concentrations were anchored to the mean detection thresholds obtained from the psychometric functions in the previous experiments: EC_{50} + 0.25 log units, EC_{50} + 0.45 log units, and EC_{50} + 0.65 log units for each salt at each temperature. This anchoring strategy ensured that NaCl and KCl concentrations occupied overlapping positions on their respective psychometric functions, rendering stimulus intensity an unreliable cue for discrimination and isolating quality-based differences. Training followed the same four-phase protocol described for detection tasks, with all training conducted at room temperature. Starting in the second training phase, mice were presented with the highest concentrations they would encounter during testing; those corresponding to EC_{50} + 0.65 log units at 14°C (NaCl: 0.11 M; KCl: 0.84 M). Advancement criteria between phases were identical to those used in detection tasks. During the testing phase, each mouse was tested at both 14°C and 36°C, serving as its own control. The three concentration pairs for each salt were presented pseudorandomly within each session. Testing alternated between temperatures across four daily sessions, with

the starting temperature counterbalanced across mice. At the conclusion of testing, mice completed two water control sessions in which all six stimulus lines contained water, with lines designated as "NaCl" or "KCl" to verify that performance returned to chance level (~50%).

Discrimination performance in *Experiment 1* was analyzed using a two-way repeated-measures ANOVA with temperature (14°C and 36°C) and concentration (EC_{50} + 0.25, 0.45, 0.65 log units) as within-subjects factors. Following a significant main effect of temperature, post hoc pairwise comparisons were conducted using paired *t* tests with Bonferroni correction to assess temperature effects within each concentration level.

Experiment 2: between-subjects design with temperature exposure from initial training. Ten mice were assigned to one of the two groups and trained to discriminate NaCl from KCl exclusively at either 14°C or 36°C throughout the entire experimental protocol. The concentration used for both training and testing was EC_{50} + 0.65 log units at the assigned temperature (14°C: 0.11 M NaCl, 0.84 M KCl; 36°C: 0.05 M NaCl, 0.42 M KCl). Side port assignments were counterbalanced between males and females within each temperature group.

The initial two training days followed the same habituation protocol described for other operant tasks. Training then proceeded through side port association and performance stabilization phases over four additional days (*days 1–4*; side port/block), with all mice completing an equal number of trials regardless of temperature group. Critically, during these training phases, mice experienced salt stimuli at their assigned testing temperature (14°C or 36°C) to ensure maximal exposure and potential adaptation to temperature conditions. The number of trials increased progressively on training days (*day 1*: 36 trials; *day 2*: 48 trials; *day 3*: 72 trials; and *day 4*: 96 trials), with a correction procedure initially implemented (*days 1* and *2*) and then removed (*days 3* and *4*).

Following the training phase, mice entered a 10-day testing period with daily sessions. All trials presented NaCl and KCl pseudorandomly at the assigned temperature. Discrimination performance in *Experiment 2* was analyzed using a linear mixed-effects model with temperature (14°C and 36°C) as a between-subjects factor, day (1–10) as a within-subjects factor, and mouse as a random factor to account for repeated measurements across testing days. The model assessed whether temperature groups differed in overall performance and whether this difference changed across days (temperature × day interaction). Following a significant temperature × day interaction, post hoc pairwise comparisons were conducted using estimated marginal means with Bonferroni correction applied family-wise across all 10 testing days to assess when temperature differences emerged and stabilized.

RESULTS

Temperature Preference Dominates Brief-Access Responses to Salt Stimuli

To examine how stimulus temperature affects the motivational properties of sodium and nonsodium salts, we conducted brief-access taste tests in water-deprived mice (Fig. 1;

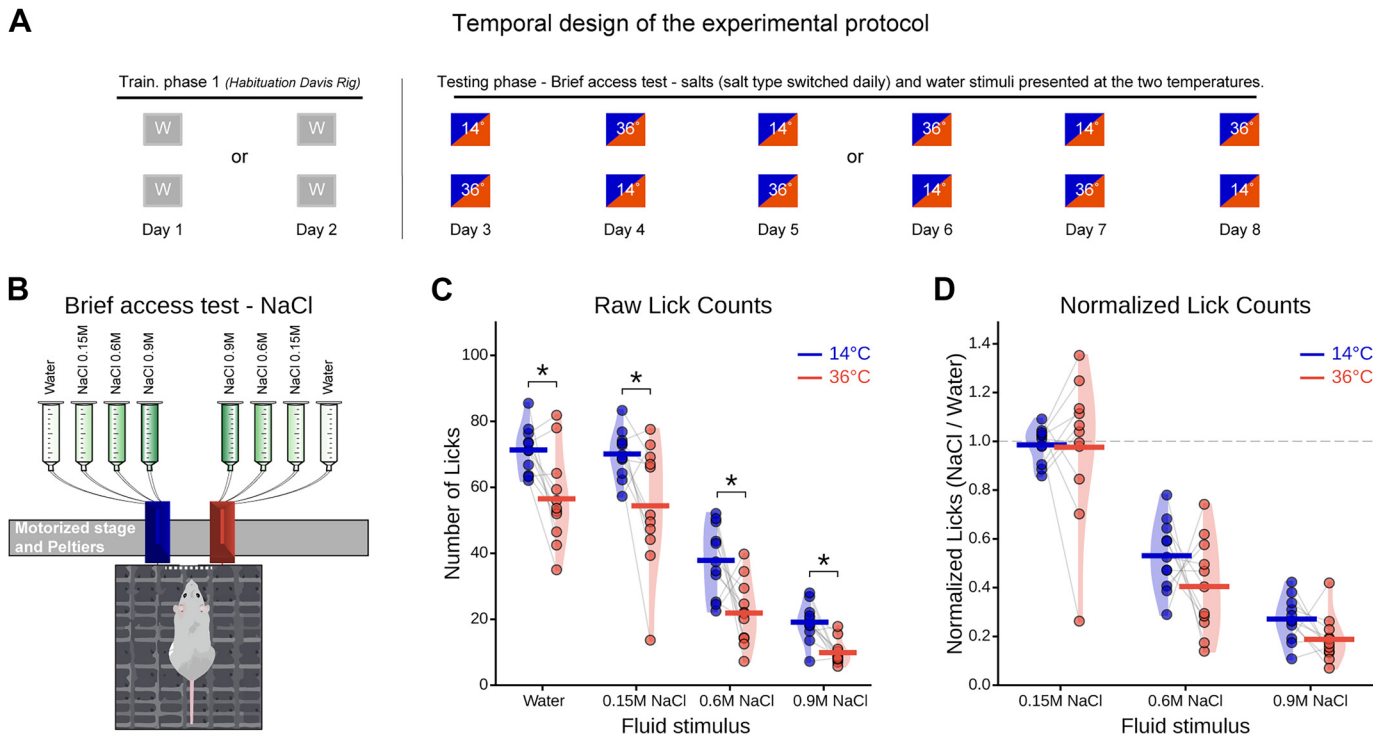

Figure 1. Temperature effects on NaCl licking behavior as a function of concentration. *A*: temporal design of the experimental protocol. *B*: schematic of the brief-access behavioral apparatus and test design. *C*: raw lick counts for water and each NaCl concentration at 14°C (blue) and 36°C (red). Each circle represents an individual mouse ($n = 11$); gray lines connect responses from the same mouse across temperatures. Horizontal lines indicate group means; shaded regions show data distributions as half violin plots. Asterisks denote significant temperature differences (post hoc pairwise comparisons using estimated marginal means with Bonferroni correction; $P < 0.04$). *D*: normalized lick counts (licks to each NaCl concentration divided by licks to water at the corresponding temperature). Data representation as in *B*. Dashed horizontal line at 1.0 indicates equal licking to water and NaCl.

see METHODS for details on timeline). In this paradigm, the animals were presented with 10-s access trials to NaCl or KCl solutions at cool (14°C) or warm (36°C) temperatures in a range of concentrations (Figs. 1*B* and 2*A*). Brief-access procedures minimize postingestive influences and provide a measure of the immediate hedonic value of taste stimuli, reflecting both appetitive and consummatory components of motivation (see Ref. 25). We first examined responses to NaCl across three concentrations (0.15, 0.6, and 0.9 M) known to span mild to strongly aversive ranges (Fig. 1, *B*–*D*). As expected, both cool and warm NaCl solutions showed concentration-dependent suppression of licking, with higher concentrations causing fewer licks, consistent with the known aversive properties of hypertonic salt solutions (26). However, a striking temperature effect emerged: mice showed an increase lick count for cool stimuli that was independent of NaCl concentration (Fig. 1*C*). A two-way repeated-measures ANOVA revealed significant main effects of both temperature ($F_{1,9} = 17.99$, $P = 0.002$) and concentration ($F_{3,27} = 177.1$, $P < 0.001$). Post hoc pairwise comparisons using estimated marginal means with Bonferroni correction revealed that water at 14°C elicited significantly more licks than water at 36°C (Fig. 1*C*; $P = 0.001$). This temperature-driven preference persisted in all NaCl concentrations tested, with mice consistently licking more to cool NaCl solutions compared with their warm counterparts (0.15 M: $P = 0.0006$; 0.6 M: $P = 0.0005$; and 0.9 M: $P = 0.037$).

To determine whether temperature specifically modulated the hedonic evaluation of NaCl itself, we normalized the lick responses to each salt concentration by dividing by the corresponding licks for water at each temperature for each animal (Fig. 1*D*). This normalization controls the baseline temperature preference and isolates responses driven by the NaCl stimulus per se. The normalized data revealed no effect of temperature on the relative aversiveness of NaCl. A two-way repeated-measures ANOVA showed a significant main effect of concentration ($F_{2,18} = 177.4$, $P < 0.001$) but no effect of temperature ($F_{1,9} = 1.11$, $P = 0.317$). Post hoc pairwise comparisons using estimated marginal means with Bonferroni correction confirmed that the magnitude of lick suppression to each NaCl concentration was comparable between 14°C and 36°C conditions (all $P > 0.05$).

Next, we tested whether this temperature preference extended to a nonsodium salt (KCl), which shares one qualitative component of NaCl taste but differs in ionic composition (27–29). Mice were tested with three concentrations of KCl (0.2 M, 0.6 M, and 0.8 M) at both temperatures. As with NaCl, mice showed a strong preference for cool over warm stimuli. A two-way repeated-measures ANOVA revealed significant main effects of both temperature ($F_{1,6} = 19.06$, $P = 0.003$) and concentration ($F_{3,18} = 62.70$, $P < 0.001$). Post hoc pairwise comparisons using estimated marginal means with Bonferroni correction showed that cool water elicited more licks than warm water (Fig. 2*B*; $P = 0.022$). This temperature bias was significant at the lowest KCl concentration (0.2 M: $P = 0.005$), with a trend toward a cool preference at higher concentrations that did not reach significance (0.6 M: $P = 0.057$; 0.8 M: $P = 0.162$). KCl solutions produced concentration-dependent lick suppression at both temperatures. When responses were normalized to water (Fig. 2*C*), the relative aversiveness of KCl did not show a systematic temperature effect. A two-way repeated-measures ANOVA showed a significant main effect of concentration ($F_{2,12} = 46.20$, $P < 0.001$) but no effect of temperature ($F_{1,6} = 1.98$, $P = 0.202$), mirroring the pattern observed with NaCl.

Together, these findings demonstrate that water-deprived mice exhibit a powerful motivational bias for cool stimuli that overwhelms any potential temperature-specific effect on salt palatability in the brief-access paradigm. The persistence of this temperature preference across water, NaCl, and KCl, regardless of concentration or ionic identity, suggests that, similarly to what has been observed in rats (15), oral temperature itself serves as a prominent hedonic signal. Due to the fact that brief-access responses reflect both the

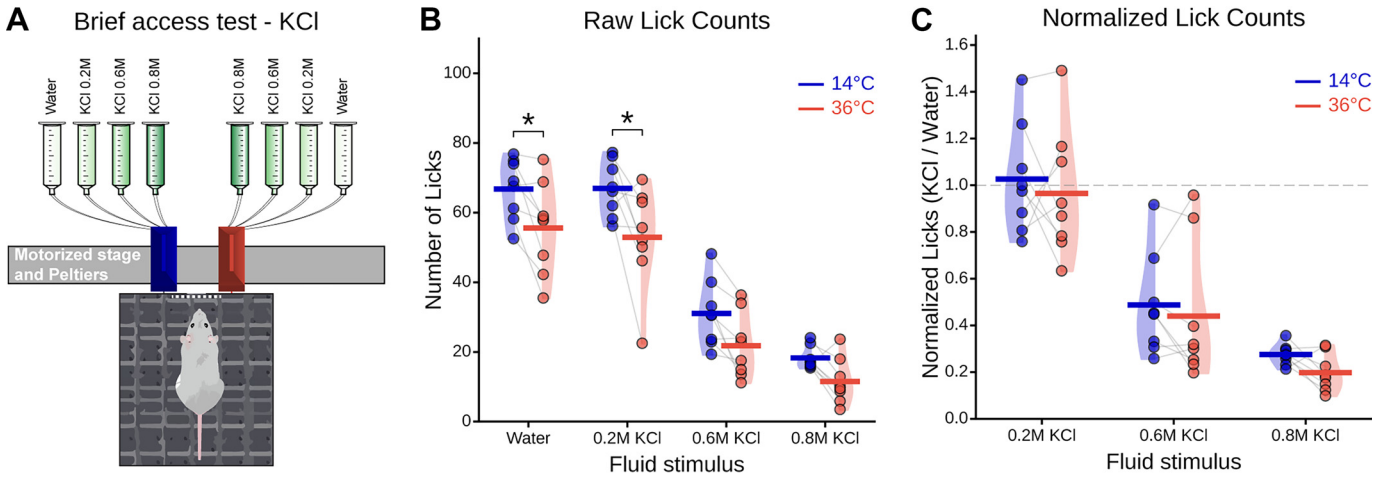

Figure 2. Temperature effects on KCl licking behavior as a function of concentration. *A*: schematic of the brief-access behavioral apparatus and task design. *B*: raw lick counts for water and each KCl concentration at 14°C (blue) and 36°C (red). Each circle represents an individual mouse ($n = 8$); gray lines connect responses from the same mouse across temperatures. Horizontal lines indicate group means; shaded regions show data distributions as half violin plots. Asterisks denote significant temperature differences (post hoc pairwise comparisons using estimated marginal means with Bonferroni correction; $P < 0.02$). *C*: normalized lick counts (licks to each KCl concentration divided by licks to water at the corresponding temperature). Data representation as in *B*. Dashed horizontal line at 1.0 indicates equal licking to water and KCl.

motivation to approach the stimulus (appetitive component) and the immediate oral-motor response upon contact (consummatory component) (25), we were unable to determine from these data whether the stimulus temperature preference primarily reflects improved appetitive drive toward cool stimuli, altered consummatory responses, or both.

To isolate the appetitive component of the temperature preference, we used a progressive ratio task (Fig. 3*A*; see METHODS for details on timeline). In the testing sessions, water-deprived mice were required to perform an operant response (licking a dry spout) to receive a 4-μL drop of water at 14°C or 36°C (Fig. 3*B*). The response requirement progressively increased (by 3 licks per trial) until the mice reached a breaking point and stopped responding. This breakpoint reflects the efficacy of the reinforcer to drive responding and provides a measure of appetitive motivation independent of consummatory responses while obviating satiation (25). Mice reached significantly higher breakpoints for cool water compared with warm water (Fig. 3*C*; paired *t* test: $t_{11} = 6.43$, $P < 0.001$, Cohen's $d = 1.86$). This large effect size indicates that cool water possesses substantially greater reinforcing efficacy than warm water under conditions of water deprivation. These findings confirm that the temperature preference observed in brief-access tests reflects a robust appetitive bias: like rats (30), water-deprived mice are willing to work considerably harder to obtain cool fluids compared with warm fluids, independent of any differences in consummatory responses. Importantly, this appetitive preference for cool temperature occurs regardless of the presence or identity of taste stimuli, explaining why temperature effects dominated responses in the brief-access paradigm and obscured any potential temperature-specific modulation of salt palatability.

Operant Conditioning Tasks Reveal Thermal Effects on Sensory-Discriminative Properties of Salt

The brief-access and progressive ratio experiments demonstrated that water deprivation induces a powerful appetitive bias for cool stimuli that dominates behavioral responses independent of stimulus identity. Although these findings clarify the motivational dynamics underlying temperature preference, they preclude any conclusions about whether temperature specifically modulates the sensory-discriminative properties of salt taste. In other words, it remains to be discerned whether temperature affects the perceived intensity and quality of salt solutions themselves.

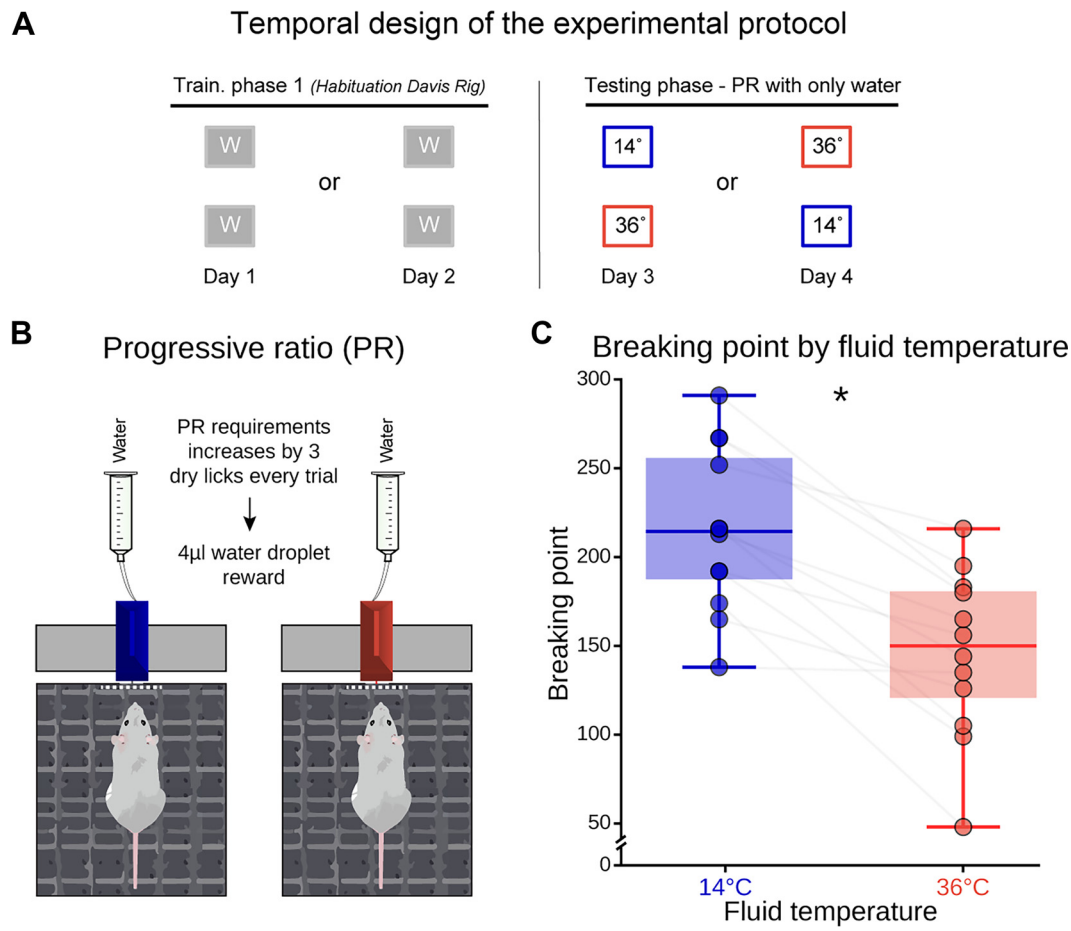

Figure 3. Temperature effects on appetitive motivation measured by progressive ratio task. *A*: temporal design of the experimental protocol. *B*: schematic of the progressive ratio (PR) task. Mice performed dry-spout licking to earn water rewards at 14°C or 36°C, with response requirements increasing by three licks per trial until reaching breakpoint. *C*: breaking points at 14°C (blue) and 36°C (red). Each circle represents an individual mouse ($n = 12$); gray lines connect responses from the same mouse across temperatures. Horizontal lines indicate group means; shaded regions show data distributions as half violin plots. Asterisk denotes significant difference between temperatures (Wilcoxon rank sum test; $P < 0.001$).

To directly assess the effects of stimulus temperature on salt perception, we used operant conditioning tasks in which taste stimuli served as discriminative cues to obtain water rewards (31–33). In these procedures, behavioral responses are controlled by reinforcement contingencies rather than the inherent hedonic properties of the stimuli (25). Operant tasks permit independent assessment of detection (sensitivity/intensity) and discrimination (quality) functions, allowing us to determine whether temperature selectively affects one or both dimensions of sensory-discriminative processing of salt taste.

Warm Temperatures Increase Salt Detection Sensitivity

To investigate whether temperature affects salt detection, we trained separate groups of mice to detect the presence of NaCl or KCl against water at either 14°C or 36°C in a two-response operant detection task (Fig. 4*A*; see METHODS for details on timeline). In this well-established psychophysical paradigm (20, 24, 34), mice sampled a central stimulus port and indicated whether the stimulus was salt or water by licking one of the two side reward ports (Fig. 4*B*). Following training at suprathreshold concentration, mice were tested in a range of concentrations to generate psychometric functions relating stimulus concentration to detection accuracy. Performance was fit with a logistic function to extract three key parameters: asymptotic performance (parameter a, reflecting maximum performance at the highest salt concentrations), psychometric slope (parameter b, reflecting the steepness of the concentration-response function), and detection threshold (parameter c, EC_{50}, the concentration at which performance reaches half-asymptotic performance) (Fig. 4, *C* and *D*). Importantly, parameters b and c were anchored to each animal's maximum performance (a), which was consistently high and independent of temperature for both NaCl and KCl (Fig. 4*E*; NaCl: Mann–Whitney $W = 38$, $P = 0.402$; KCl: Mann–Whitney $W = 57$, $P = 0.641$). This demonstrates that stimulus temperature alone did not affect the ability of the animal to perform the task.

For NaCl, mice tested at 36°C exhibited significantly lower detection thresholds compared with mice tested at 14°C (Fig. 5*A*; $t_{18} = 2.43$, $P = 0.026$, Cohen's $d = 1.09$). Converting from logarithmic to linear units, this represents threshold concentrations of ∼24 mM at 14°C versus 10 mM at 36°C, a 2.4-fold increase in sensitivity at warmer temperatures. The psychometric slope was also significantly steeper at 14°C compared with 36°C ($t_{18} = -2.13$, $P = 0.047$, Cohen's $d = -0.96$), indicating a sharper transition from chance to asymptotic performance at cooler temperatures.

KCl detection showed a similar pattern (Fig. 5*B*). Although the 14°C and 36°C average psychometric curves overlapped at the lowest concentrations and diverged primarily at intermediate concentrations (10^{-2}; Fig. 4*D*), detection thresholds were significantly lower at 36°C compared with 14°C (Mann–Whitney $W = 103$, $P = 0.020$), corresponding to threshold concentrations of ∼186 mM versus 93 mM. The psychometric slopes were significantly steeper at 14°C than at 36°C ($t_{14.6} = -2.91$, $P = 0.011$, Cohen's $d = -1.09$).

These findings reveal a robust effect of stimulus temperature on salt detection: warm temperatures significantly enhance perithreshold sensitivity to both sodium and nonsodium salts, lowering detection thresholds by approximately twofold. This represents a clear dissociation from the motivational findings; although cool temperatures are strongly preferred in brief-access tests and elicit greater effort in progressive ratio tasks, warm temperatures increase the sensory salience of salts, making both NaCl and KCl more detectable at lower concentrations. These findings demonstrate that temperature differentially affects motivational responses and sensory detection: although cool temperatures drive appetitive behavior in general, warm temperatures enhance sensitivity salt detectability, underscoring the importance of assessing temperature effects across multiple taste dimensions. Importantly, enhancement of salt detection at warm temperatures occurred for both NaCl and KCl, indicating that this may be a general property of the influence of stimulus temperature on salt taste intensity rather than a sodium-specific effect.

Warm Temperatures Reduce Discriminability between NaCl and KCl

The detection experiments demonstrated that warm temperatures enhance salt sensitivity, lowering perceptual thresholds for both NaCl and KCl (Figs. 4 and 5). However, detecting a single stimulus can be different from distinguishing between two stimuli. Detection performance is primarily linked to the intensity dimension (prothetic; how strong is the stimulus?), whereas discrimination engages the quality dimension (metathetic; what is the stimulus?) (35). Critically, any manipulation that affects detection thresholds, such as varying stimulus temperature, could alter the perceived intensity of stimuli, creating a confound when assessing quality-based discrimination. To isolate the effects of temperature on quality discrimination independent of intensity, we used a "concentration anchoring" strategy (Fig. 6). For each temperature condition, we selected three concentrations of NaCl and three concentrations of KCl based on the mean detection thresholds (EC_{50} values) obtained in the detection experiments: EC_{50} + 0.25 log units, EC_{50} + 0.45 log units, and EC_{50} + 0.65 log units. This approach ensures that NaCl and KCl concentrations occupy overlapping positions along each temperature-specific psychometric function, rendering stimulus intensity an unreliable cue for discrimination (Fig. 6). With intensity controlled, any differences in discrimination performance between temperatures must reflect changes in the qualitative signatures of the two salts. Having established the concentration-anchoring strategy, we conducted two complementary discrimination experiments to assess whether temperature affects the ability to distinguish NaCl from KCl when intensity cues are controlled (Fig. 7, *A*–*C*).

In the first experiment, mice were trained with salt stimuli at room temperature to discriminate between the highest concentrations of NaCl and KCl until they reached stable performance (>85% correct for two consecutive days; Fig. 7*B*, Train. Phase 1 and 2). During the testing phase, the same mice were tested at both 14°C and 36°C in all three anchored concentration pairs (EC_{50} + 0.25, 0.45, 0.65 log units), allowing each animal to serve as its own control (within-subjects design). The testing sessions alternated between temperatures over 4 days, with the starting temperature counterbalanced between mice. Water control sessions verified that performance was not driven by nongustatory

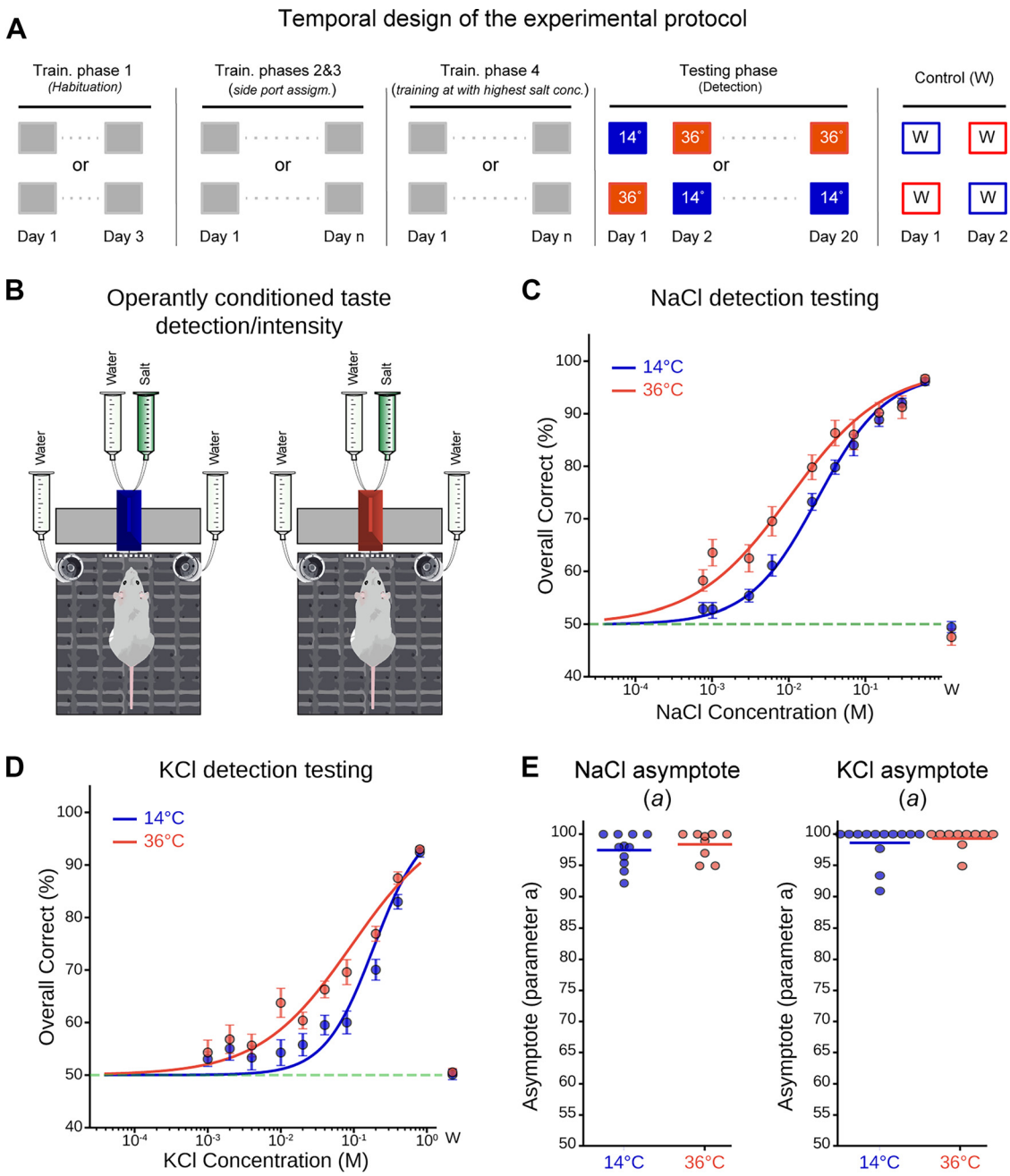

Figure 4. Temperature effects on salt detection sensitivity. *A*: temporal design of the experimental protocol. *B*: schematic of the two-response operant detection task. Mice learned to discriminate a salt stimulus (NaCl or KCl) from water at a specific temperature by choosing the correct side port to receive water reinforcement. *C*: NaCl detection performance as a function of concentration at 14°C (blue) and 36°C (red). Each point represents means ± SE across mice (14°C: $n = 11$; 36°C: $n = 9$). Solid curves show psychometric functions fit to the data. Green dashed line indicates chance performance (50%). Water control performance (W) is shown at the right. *D*: KCl detection performance as a function of concentration. Data representation as in *B* (14°C: $n = 13$; 36°C: $n = 10$). *E*: NaCl and KCl asymptotic performances at each temperature. Each circle represents an individual mouse; horizontal lines indicate group means.

cues. Mice discriminated NaCl from KCl significantly better at 14°C compared with 36°C in all concentration pairs (peri- to suprathreshold) tested (Fig. 7*D*). A two-way repeated-measures ANOVA revealed significant main effects of both temperature ($F_{1,12} = 147.9$, $P < 0.001$) and concentration ($F_{2,24} = 56.42$, $P < 0.001$), along with a significant interaction ($F_{2,24} = 5.02$, $P = 0.015$). Post hoc paired t tests with Bonferroni correction confirmed that cool stimulus temperatures supported superior discrimination at all concentration levels: EC_{50} + 0.25 log ($t_{12} = 6.73$, $P < 0.001$, Cohen's $d = 1.87$); EC_{50} + 0.45 log ($t_{12} = 9.77$, $P < 0.001$, $d = 2.71$); and EC_{50} + 0.65 log ($t_{12} = 12.48$, $P < 0.001$, $d = 3.46$). The large effect sizes in all concentrations underscore the robust impact of temperature on salt quality discrimination. Even at the highest concentration tested (EC_{50} + 0.65 log units), where both salts were well

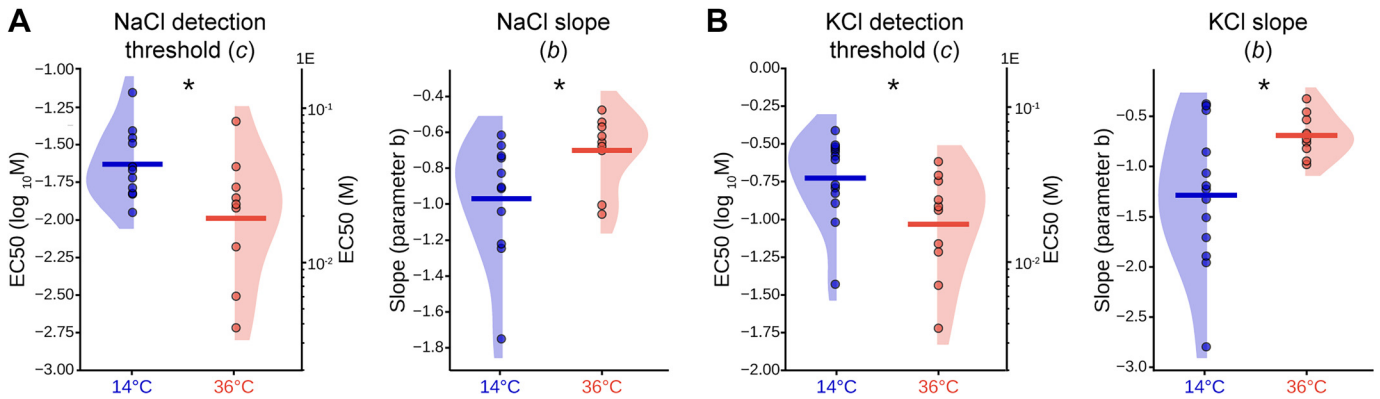

Figure 5. Temperature effects on salt detection sensitivity. *A*: NaCl detection thresholds (*left*, parameter *c*, EC_{50}) and psychometric slopes (*right*, parameter *b*) at each temperature. Each circle represents an individual mouse; horizontal lines indicate group means; shaded regions show data distributions as half violin plots. Asterisks denote significant differences between temperatures (independent *t* tests). *B*: KCl detection thresholds and slopes. Data representation as in *A*. Asterisks denote significant differences (threshold: Mann–Whitney *U* test; slope: Welch's *t* test; $P < 0.05$).

above the detection threshold and discrimination at 14°C approached the ceiling performance (89%), warm temperatures still reduced the accuracy to ~67%. Water control trials confirmed that performance was at chance level when all lines contained water (Fig. 7*D*, *rightmost*), demonstrating that mice relied on gustatory cues rather than nongustatory sensory information.

To confirm and extend the findings of *Experiment 1*, a separate cohort of mice was trained and tested exclusively at 14°C or 36°C throughout the experimental protocol (Fig. 7*C*). In this between-subjects design, separate groups experienced their assigned temperature from the initial habituation phase onward. Mice were trained on the highest concentration pair (EC_{50} + 0.65 log units) for up to 5 days, then entered a 10-day testing phase at the same concentration. Consistent with *Experiment 1*, mice trained and tested at 14°C exhibited superior discrimination performance compared with mice at 36°C throughout the testing phase (Fig. 7*E*). A linear mixed-effects model revealed a significant temperature × day interaction ($F_{1,88} = 20.20$, $P < 0.001$), indicating that the performance difference between temperatures increased progressively across days. The temporal dynamics revealed a striking and sustained divergence: the 14°C group rapidly achieved and maintained high discrimination precision, whereas the 36°C group remained at substantially lower performance levels (~70%) with greater variability across days. Post hoc pairwise comparisons using estimated marginal means with Bonferroni correction (family-wise throughout all 10 days) confirmed that temperature differences emerged as significant on *day 4* ($P = 0.025$) and became highly significant throughout *days 5–10* (*day 5:* $P = 0.008$; *day 6:* $P = 0.003$; *days 7–10*: all $P < 0.001$), with a final performance difference of ~24% on *day 10* ($P < 0.001$). This sustained performance divergence reflects a fundamentally easier discriminability between NaCl and KCl at cool temperatures rather than a merely slower acquisition. The fact that mice tested at 36°C never approached the performance levels achieved by mice

A

Salts concentration anchored by the mean detection threshold (EC_{50}) at 14°C.

	$Log_{10}M$		Linear [M]	
	NaCl	*KCl*	*NaCl*	*KCl*
EC_{50} + 0.25log_{10}	-1.37	-0.47	0.04	0.33
EC_{50} + 0.45log_{10}	-1.17	-0.27	0.07	0.53
EC_{50} + 0.65log_{10}	-0.97	-0.77	0.11	0.84

B

Salts concentration anchored by the mean detection threshold (EC_{50}) at 36°C.

	Log_{10}[M]		Linear [M]	
	NaCl	*KCl*	*NaCl*	*KCl*
EC_{50} + 0.25log_{10}	-1.73	-0.78	0.02	0.17
EC_{50} + 0.45log_{10}	-1.53	-0.58	0.03	0.26
EC_{50} + 0.65log_{10}	-1.33	-0.38	0.05	0.42

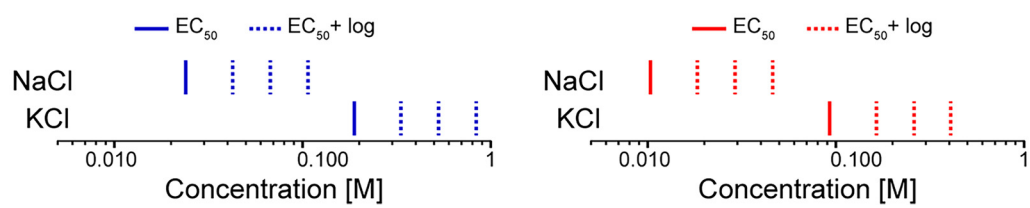

Figure 6. Concentration-anchoring strategy for discrimination experiments. *A*: concentration selection for 14°C. *Top*: table showing NaCl and KCl concentrations (logarithmic and linear) anchored to mean detection thresholds (EC_{50}) obtained from detection experiments. *Bottom*: schematic showing concentration ranges for each salt. Solid lines indicate mean EC_{50}; dashed lines indicate the three test concentrations (EC_{50} + 0.25, 0.45, and 0.65 log units). *B*: concentration selection for 36°C. Data representation as in *A*. This anchoring strategy ensured that NaCl and KCl occupied overlapping positions along their respective psychometric functions at each temperature, rendering intensity an unreliable cue for discrimination.

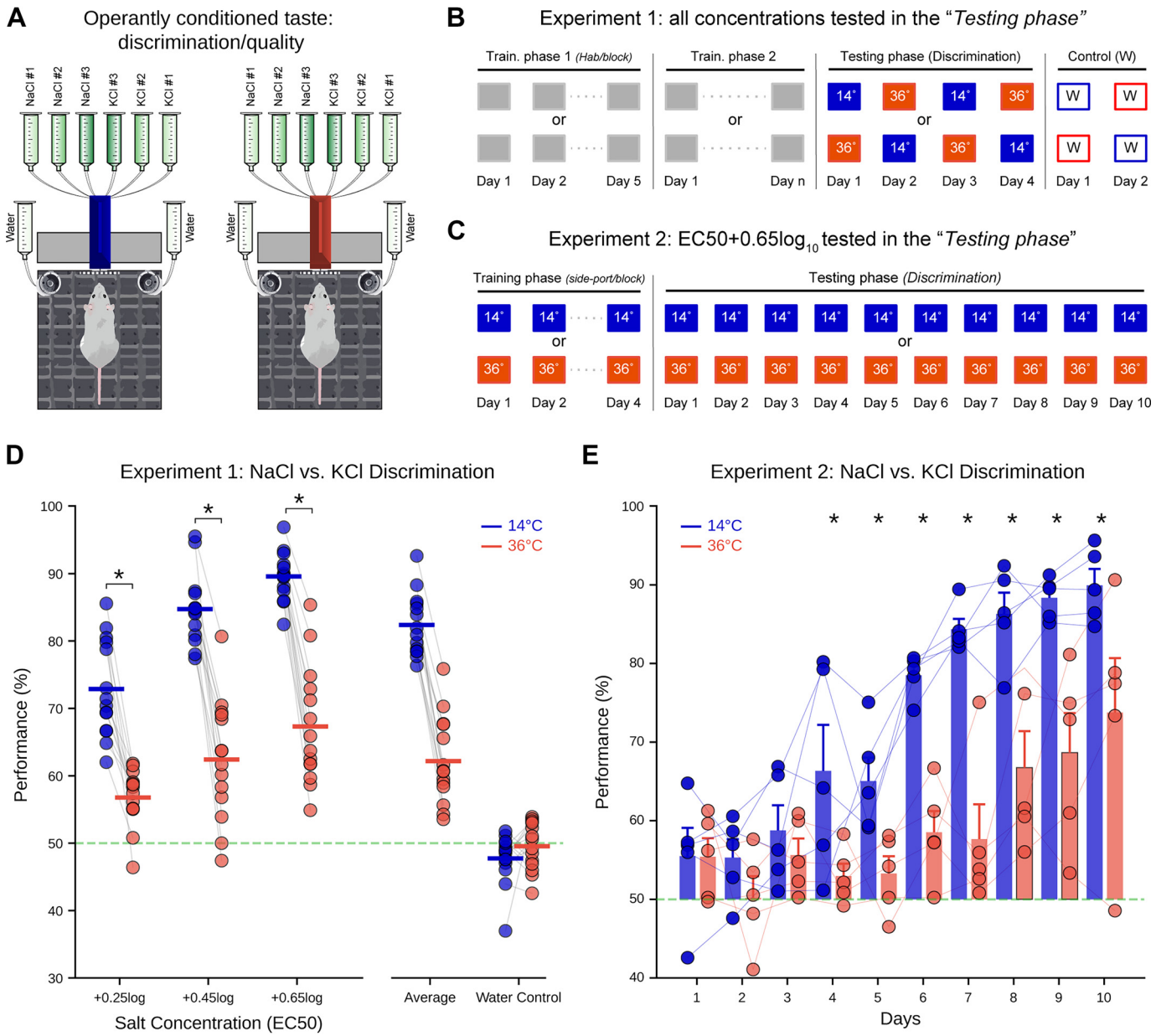

Figure 7. Temperature effects on NaCl vs. KCl discrimination. *A*: schematic of the two-response operant discrimination task. Mice learned to distinguish NaCl from KCl at a specific temperature by choosing the correct side port to receive water reinforcement. *B*: experimental design for *Experiment 1* (within-subjects). Mice were tested at both 14°C and 36°C across three intensity-matched concentration pairs (EC_{50} + 0.25, 0.45, and 0.65 log units). Color squares indicate stimulus temperature (gray: room temperature during training; blue: 14°C; red: 36°C). Rows show counterbalanced testing order. *C*: experimental design for *Experiment 2* (between-subjects). Separate groups were trained and tested exclusively at either 14°C (blue) or 36°C (red) using a single concentration pair (EC_{50} + 0.65 log units) throughout all phases. *D*: discrimination performance in *Experiment 1* as a function of concentration at 14°C (blue) and 36°C (red). Each circle represents an individual mouse ($n = 13$); gray lines connect responses from the same mouse across temperatures. Horizontal lines indicate group means; shaded regions show data distributions as half violin plots. Average performance across all concentrations and water control performance are shown at right. Green dashed line indicates chance performance (50%). Asterisks denote significant differences (paired *t* tests with Bonferroni correction; $P < 0.001$). *E*: discrimination performance in *Experiment 2* across 10 testing days. Each circle represents an individual mouse (14°C: $n = 5$; 36°C: $n = 5$); bars show group means ± SE; shaded regions show data distributions as half violin plots. Green dashed line indicates chance performance. Asterisks denote days with significant temperature differences (linear mixed-effects model with Bonferroni correction across all 10 days; $P < 0.03$).

tested at 14°C, even within the 10-day temporal window tested here, confirms the results observed in *Experiment 1* (Fig. 7*D*): the stimulus temperature modulates the intrinsic qualitative distinctiveness of the two salts. At 36°C, NaCl and KCl become perceptually more similar, making the discrimination task inherently more difficult regardless of concentration level (*Experiment 1*) or extended exposure (*Experiment 2*).

Together, the detection and discrimination experiments reveal a complex and multidimensional influence of stimulus temperature on salt taste. Warm temperatures exert opposing effects on different sensory-discriminative dimensions; they

enhance detection sensitivity (lowering thresholds ~2-fold for both NaCl and KCl) while simultaneously reducing the qualitative discriminability between the two salts. This dissociation demonstrates that temperature differentially modulates the prothetic dimension of taste (intensity: how strong is it?) versus the metathetic dimension (quality: what is it?). At 36°C, both sodium and nonsodium salts become more intense at lower concentrations but perceptually more similar in quality, making them harder to distinguish even when intensity is controlled.

DISCUSSION

Prior investigations of temperature effects on salt taste have yielded inconsistent findings. Human psychophysical data range from reports of enhanced NaCl intensity at warm temperatures (16) to no effect (17), whereas threshold studies have suggested optimal sensitivity at moderate temperatures (22–32°C) (36, 37). Electrophysiological studies in anesthetized rodents have revealed concentration-dependent effects: low NaCl concentrations elicit maximal chorda tympani responses at cool temperatures (~10°C–20°C), whereas such responses to higher concentrations peak at warmer temperatures (~30°C) (38, 39). Recent neurophysiological data in anesthetized mice have shown that cooling, but not warming, enhances nucleus of the solitary tract responses to perithreshold NaCl (4). Given that our detection thresholds fall within concentration ranges previously shown to be optimally detected at cool or moderate temperatures, these findings would predict enhanced sensitivity at 14°C. Contrary to this expectation, we observed that warm stimulus temperatures (36°C) consistently enhanced detection for both NaCl and KCl by approximately twofold. Other than the data already discussed (14, 15), to our knowledge, no studies in rodents have systematically evaluated how stimulus temperature affects the different functions of taste across qualities. In humans, warming has been reported to enhance the perceived intensity of sweet (17, 40) and, to a lesser extent, bitter stimuli (17), whereas effects on sourness are weaker and less consistent (17, 36); these human findings are in any case difficult to situate within a functional framework, as they combine psychophysically distinct measures—detection, intensity, and hedonics—that tap dissociable taste functions and cannot be directly compared. Accordingly, this is, to our knowledge, the first study to provide a comprehensive evaluation of the effects of stimulus temperature across the different domains of taste behavior in rodents.

Our results reveal a profound effect of stimulus temperature on the motivational/hedonic aspect—which includes both appetitive and consummatory behaviors (25)—of salt taste. Water-deprived mice showed a strong avidity for cool (14°C) over warm (36°C) stimuli in brief-access tests, regardless of salt identity, concentration, or even in the absence of salt (water alone). Progressive ratio testing confirmed that this effect can be demonstrated by assessing purely appetitive behavior; mice were willing to exert considerably greater effort to obtain cool fluids compared with warm fluids. This robust motivational effect, consistent with prior observations in water-deprived rats (15, 30), demonstrates that oral stimulus temperature can powerfully influence—and potentially confound—the interpretation of any taste-related behavioral readout that relies on water restriction and is driven by the appetitive and consummatory aspects of the taste stimulus, as is the case with the brief-access test in rodents. In addition, these results align with human studies where cool water is rated as more rewarding and effectively quenches thirst during dehydration (41, 42). Indeed, this thirst-quenching effect can be elicited by perceived oral coldness even in the absence of physical cooling, such as through the chemical activation of TRPM8 receptors (43), suggesting that cold-sensitive oropharyngeal signaling provides a potent homeostatic signal of satiation.

Nevertheless, these shifts in motivational state do not fully account for the alterations in taste processing observed in our sensory-discriminative tasks. By using a paradigm where reinforcement contingencies, rather than hedonic drive, guide the animal's behavior (25), we were able to demonstrate that stimulus temperature fundamentally changes salt taste perception across distinct sensory dimensions. Specifically, warm temperatures exert opposing effects on the prothetic dimension of taste (intensity: how strong is it?) versus the metathetic dimension (quality: what is it?). Although 36°C enhanced detection sensitivity for both NaCl and KCl, lowering thresholds approximately twofold and making both salts perceptually more intense at lower concentrations, it simultaneously impaired qualitative discrimination between them. This impairment occurred despite our concentration-anchoring strategy, in which NaCl and KCl concentrations were matched to their respective detection thresholds at each temperature (EC_{50} + 0.25, 0.45, 0.65 log units). Even if the respective concentrations of each salt were not completely isointense, this approach ensured that both salts occupied overlapping positions on their psychometric functions, rendering intensity an unreliable cue and forcing reliance on qualitative differences. The robust discrimination impairment at warm temperatures, observed across both within-subjects and between-subjects designs, reveals that even when intensity is controlled, sodium and nonsodium salts become perceptually more similar at 36°C. This temperature-induced modification of salt taste perception could provide insights into the complex and still incompletely understood amiloride-insensitive salt taste pathway, as well as inform salt substitution strategies in foods served at elevated temperatures. It would be informative to compare our psychophysical detection thresholds and NaCl–KCl discrimination at 14°C and 36°C with the values available in the literature for mice tested at room temperature. Although one must be circumspect in directly comparing values obtained in different preparations, methods, and laboratories, two points are worth noting. With respect to sensitivity, our detection thresholds at 36°C are, to our knowledge, lower than any previously reported room-temperature thresholds in mice for both NaCl (44–48) and KCl (27). With respect to discrimination, only one study has assessed NaCl–KCl discrimination in mice, using isomolar concentrations of the two salts rather than the threshold-anchored concentrations used here (27); nevertheless, that study reported high discriminability between the two salts at room temperature, comparable to what we observed at 14°C.

Beyond these practical implications, the opposing thermal effects on salt detection and discrimination raise the question of their physiological origin. Stimulus temperature can both activate thermal sensors and modulate the gating and

conductance of transduction channels, so the behavioral shifts we observe may reflect direct effects on taste receptor cells, cross-modal integration of trigeminal thermal and gustatory signals at peripheral and central stages of processing, or a combination of the two. Our data offer one constraint at the peripheral level: because KCl—which is largely amiloride-insensitive and does not rely on ENaC (27)—showed thermal modulation equivalent to NaCl, a sodium-specific, ENaC-based account (49, 50) cannot fully explain the enhanced sensitivity at 36°C. This parallel instead seems to implicate nonselective, warmth-sensitive transduction pathways, common to both salts. Identifying the mechanisms involved—peripheral, central, or both—and how their recruitment at warm temperatures both sharpens detection and blurs discrimination between salts, will require further investigation.

Several technical considerations merit discussion. Our choice of test temperatures (14°C and 36°C) was guided by multiple factors. Both temperatures fall outside the range of overtly noxious thermal stimuli (21, 22), and they are sufficiently separated to allow initial investigation of thermal effects before examining finer temperature differences. Future work exploring temperatures closer to oral thermal liminal sensitivity in mice, which remain to be systematically characterized, will be necessary to determine whether these perceptual shifts represent a graded response to thermal deviation from baseline tongue temperature or a discrete phenomenon triggered at specific temperature thresholds. Our stimulus delivery approach also differs fundamentally from prior electrophysiological studies conducted in anesthetized preparations. Rather than applying large volumes that create sustained thermal gradients across the tongue surface, an approach nearly impossible to control in awake-behaving animals, our operant tasks delivered discrete 4 μL droplets at controlled temperatures. Thus, the observed effects reflect responses to brief, mostly tongue-localized thermal stimulation rather than prolonged adaptation to altered global oral temperature. These methodological differences may partly account for discrepancies between our behavioral findings and predictions based on anesthetized electrophysiological data from previous studies (51).

Our findings have implications for both the basic operational principles of gustatory neuroscience and potential translational applications. From a fundamental perspective, these results underscore that stimulus temperature is not merely an incidental feature of taste stimuli but a basic physical parameter, comparable to concentration and molecular structure, that systematically modulates perception. The fact that relatively modest stimulus temperature variations within the innocuous physiological range can alter both the intensity and quality dimensions of salt taste highlights the necessity of controlling or systematically varying temperature in gustatory research. Our results indicate that warm temperatures increase the perceptual similarity between NaCl and KCl. Although identifying effective salt substitutes is a global public health priority due to the links between sodium and cardiovascular disease (52–54), the efficacy of these substitutes often depends on their ability to mimic the "salty" profile of sodium. It is important to note that our current experiments were not designed to determine the directionality of this thermal effect, specifically, whether KCl is becoming more "sodium-like" or NaCl is becoming more "potassium-like." Future studies will delve into the specific qualitative shifts driving this convergence; however, irrespective of the directionality, these results hold significant translational relevance for sodium replacement strategies, as the narrowing of the perceptual gap at 36°C suggests that potassium-based substitutes may be more effective in foods served at warm temperatures.

DATA AVAILABILITY

Datasets generated and/or analyzed during the current study is available in the vincisLab GitHub repository (https://github.com/vincisLab).

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

M.E.M., A.C.S., and R.V. conceived and designed research; M.E.M. and A.C.S. performed experiments; M.E.M., A.C.S., and R.V. analyzed data; M.E.M. and R.V. interpreted results of experiments; M.E.M. and R.V. prepared figures; M.E.M., A.C.S., and R.V. drafted manuscript; M.E.M., A.C.S., and R.V. edited and revised manuscript; M.E.M., A.C.S., and R.V. approved final version of manuscript.

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