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Intrinsic excitability in layer IV-VI anterior insula to basolateral amygdala projection neurons correlates with the confidence of taste valence encoding

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2 amygdala projection neurons correlates with the confidence of
3 taste valence encoding

4

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15 SKC and AY led the project. AY, SKC and KR designed the research. KR supervised the research.

16 SKC, AY, HK, and MK performed the research. SKC, AY, LM, RS, FC, and SS analyzed the data.

17 AY, SKC and KR drafted the paper. All authors reviewed and contributed to the manuscript.

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50 **Data availability**

51 All data generated or analyzed during this study are included in the manuscript and supporting
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60 Authors report no conflict of interest.

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65

66 **Abstract**

67 Avoiding potentially harmful, and consuming safe food is crucial for the survival of living
68 organisms. However, the perceived valence of sensory information can change following
69 conflicting experiences. Pleasurability and aversiveness are two crucial parameters defining the
70 perceived valence of a taste and can be impacted by novelty. Importantly, the ability of a given
71 taste to serve as the conditioned stimulus (CS) in conditioned taste aversion (CTA), is dependent
72 on its valence. Activity in anterior insula (aIC) layer IV-VI pyramidal neurons projecting to the
73 basolateral amygdala (BLA) is correlated with, and necessary for CTA learning and retrieval, as
74 well as the expression of neophobia towards novel tastants, but not learning taste familiarity. Yet,
75 the cellular mechanisms underlying the updating of taste valence representation in this specific
76 pathway are poorly understood. Here, using retrograde viral tracing and whole-cell patch-clamp
77 electrophysiology in trained mice, we demonstrate that the intrinsic properties of deep-lying layer
78 IV-VI, but not superficial layer I-III aIC-BLA neurons, are differentially modulated by both
79 novelty and valence, reflecting the subjective predictability of taste valence arising from prior
80 experience. These correlative changes in the profile of intrinsic properties of LIV-VI aIC-BLA
81 neurons were detectable following both simple taste experiences, as well as following memory
82 retrieval, extinction learning and reinstatement.

83

84 **Significance statement**

85 Learning to form aversive or safe taste memories is dependent on genetic predisposition as well as
86 previous experiences. In mice, anterior insula neurons projecting to the basolateral amygdala (aIC-
87 BLA) are indispensable for learning and retrieving learned taste aversion. Kolatt Chandran et al.
88 demonstrate that the intrinsic properties of aIC-BLA neurons, represent the certainty of taste
89 valence prediction, but not percept. Predictive valence-specific changes are reflected through
90 excitability, being low when taste outcome is highly predictive (i.e., following aversive taste
91 memory retrieval or unreinforced familiarization), and high when taste valence is uncertain (i.e.,
92 following novelty or aversive taste memory extinction). In addition, the results propose a neuronal
93 mechanism underlying the long delay between taste and visceral discomfort in conditioned taste
94 aversion.

95 Introduction

96 In the natural setting, animals approach novel taste stimuli tentatively, as to closely examine them
97 according to a genetic plan, as well as in relation to associated visceral consequences (Schier and
98 Spector, 2019). Bitter and sour tastes are innately aversive, acting as warning signals for the
99 presence of toxins (Bachmanov et al., 1996). Conversely, neophobia to innately appetitive sweet
100 and moderately salty tastants dissipates over time (Lin et al., 2012). Importantly, animals can learn
101 to avoid innately appetitive tastants (e.g., saccharin-, or NaCl-water – the conditioned stimulus,
102 CS), through conditioned taste aversion - CTA (Garcia et al., 1955; Nachman and Ashe, 1973).
103 This single-trial associative learning paradigm results in robust aversion following the pairing of
104 the CS with a malaise-inducing agent (the unconditioned stimulus, US), such as LiCl (Bures et al.,
105 1998). CTA memories are robust, but can be extinguished through unreinforced CS re-exposures,
106 and subsequently reinstated through US re-exposure (Schachtman et al., 1985; Mickley et al.,
107 2004). Unlike other forms of classical conditioning, the inter-stimulus interval (ISI) between taste
108 experience (CS) and visceral outcome (US), extends to several hours (Adaikkan and Rosenblum,
109 2015). How CTA learning enables this long-trace associative process, within timeframes that
110 deviate from classical Hebbian plasticity mechanisms is currently unknown (Chinnakkaruppan et
111 al., 2014; Adaikkan and Rosenblum, 2015).

112 The primary taste cortex - the anterior insula (aIC), along with the basolateral amygdala (BLA),
113 govern the encoding and retrieval of taste information (Piette et al., 2012; Bales et al., 2015).
114 Gustatory processing in IC neurons encompasses thalamocortical and corticocortical inputs that
115 relay taste-, as well as palatability-related inputs from the BLA, that reflect the emotional valence
116 associated with taste stimuli (Stone et al., 2020). Neuronal taste responses at the IC and BLA are
117 plastic and spatially dispersed, using temporal information to encode multiple types of information

118 relating to stimulus identity and palatability (Grossman et al., 2008; Sadacca et al., 2012; Arieli et
119 al., 2020; Vincis et al., 2020). Both synaptic plasticity and neuronal intrinsic properties are
120 proposed to serve as cellular mechanisms underlying learning and memory (Citri and Malenka,
121 2008; Sehgal et al., 2013). CTA learning promotes LTP induction in the BLA-IC pathway (Jones
122 et al., 1999; Juárez-Muñoz et al., 2017), and strengthens cell-type specific functional connectivity
123 along the projection (Haley et al., 2016). Intrinsic excitability is the tendency of neurons to fire
124 action potentials when exposed to inputs, reflecting changes in the suit and properties of specific
125 ion channels (Disterhoft et al., 2004; Song and Moyer, 2018). Even though independent
126 mechanisms are involved, recent evidence indicates learning and memory necessitates the that
127 coupling of intrinsic and synaptic plasticity (Turrigiano, 2011; Greenhill et al., 2015; Wu et al.,
128 2021).

129 The IC is an integration hub tuned for the encoding of both exteroceptive as interoceptive
130 information (Gogolla et al., 2014; Haley and Maffei, 2018; Livneh et al., 2020; Koren et al., 2021).
131 By virtue of its extensive network of connectivity, this elongated cortical structure has been shown
132 to integrate sensory, emotional, motivational, and cognitive brain centers through distinct
133 mechanisms. For example, deletions of either *Fos* or *Stk11* in BLA-aIC neurons, alter intrinsic
134 properties at the aIC, and impair CTA acquisition (Levitan et al., 2020). Furthermore, approach
135 behaviors in social decision-making are modulated by subjective and sex-specific affective states
136 that regulate cell-type-specific changes in intrinsic properties at IC projections to the nucleus
137 accumbens (Rogers-Carter et al., 2018, 2019; Rieger et al., 2022). The posterior IC (pIC)
138 integrates visceral-sensory signals of current physiological states with hypothalamus-gated
139 amygdala anticipatory inputs relating to food or water ingestion, to predict future physiological
140 states (Livneh et al., 2017, 2020). Conversely, aversive visceral stimuli such as LiCl, activate

141 CaMKII neurons projecting to the lateral hypothalamus in right-, but not the left IC, whose
142 optogenetic activation or inhibition can bidirectionally regulate food consumption (Wu et al.,
143 2020). We have previously shown that the aIC-BLA projection is necessary and sufficient for CTA
144 acquisition and retrieval (Lavi et al., 2018; Kayyal et al., 2019), while CTA retrieval requires
145 activation of the projection concomitant with parvalbumin (PV) interneurons (Yiannakas et al.,
146 2021). Moreover, artificial activation of aIC-BLA projecting neurons is sufficient to induce CTA
147 for appetitive taste (Kayyal et al., 2019). Here, using retrograde viral tracing, behavioral analysis,
148 and whole-cell patch-clamp slice electrophysiology, we assessed two hypotheses: (1) That the
149 intrinsic properties of the aIC-BLA projection change as a function of certainty of taste valence
150 prediction, but not percept; and (2) that predictive valence-specific changes in intrinsic properties
151 would be reflected through excitability, being low when taste outcome is highly predictive (i.e.,
152 following CTA retrieval or unreinforced familiarization), and high when taste valence is uncertain
153 (i.e., following novelty or extinction). Our data demonstrate for the first time that the intrinsic
154 properties of LIV-VI aIC-BLA neurons are differentially regulated by innate and learned drives,
155 reflecting the confidence of currently perceived taste valence.

156

157 **Materials and methods**

158 **Animals**

159 Animals used were 8–12-week-old C57BL/6j (WT) adult male mice. Mice were kept in the local
160 animal resource unit at the University of Haifa on a 12-hour dark/light cycle. Water and chow
161 pellets were available ad libitum, while ambient temperature was tightly regulated. All procedures
162 conducted were approved by the University of Haifa Animal Care and Use Committee (Ethics

163 License 554/18), as prescribed by the Israeli National Law for the Protection of Animals –
164 Experiments with Animals (1994).

165

166 **Animal surgery and viral injections**

167 Following surgery and stereotactic injection of viral vectors, behavioral paradigms were
168 performed, as previously described (Yiannakas et al., 2021). Briefly, mice were treated with
169 norocarp (0.5mg/kg), before being anesthetized (M3000 NBT Israel/Scivena Scientific) and
170 transferred to a Model 963 Kopf® stereotactic device. Upon confirming the lack of pain responses,
171 the skull was surgically exposed and drilled to bilaterally inject 0.25µl of ssAAV_retro2-hSyn1-
172 chi-mCherry-WPRE-SV40p(A) (physical titer 8.7 x 10E12 vg/ml), at the BLA (AP -1.58; ML ±
173 3.375; DV - 4.80). Viral delivery was performed using a Hamilton micro-syringe (0.1µL/minute),
174 while the sculp was cleaned and closed using Vetbond®. Animals were then administered with
175 0.5mg/kg norocarp and 0.5mg/kg of Baytril (enrofloxacin), and then transferred to a clean and
176 heat-adjusted enclosure for 2 hours. Upon inspection, mice were returned to fresh cages along with
177 similarly treated cage-mates. Weight-adjusted doses of the Norocarp and Baytril were administered
178 for an additional 3 days. All AAV constructs used in this study were obtained from the Viral Vector
179 Facility of the University of Zurich (<http://www.vvf.uzh.ch/>).

180

181 **Electrophysiological studies of the influence of innate taste identity, novelty, and valence** 182 **on aIC-BLA excitability**

183 WT mice treated with viral constructs labeling aIC-BLA projecting neurons were used for
184 electrophysiological studies. Upon recovery, mice were randomly assigned into treatment groups

185 (Figure 1). Following 24hrs of water deprivation, animals were water restricted for 3 days,
186 receiving water in pipettes ad libitum for 20 minutes/day (Kayyal et al., 2019; Yiannakas et al.,
187 2021). This regime has been extensively used by our lab as it allows rodents to reliably learn to
188 drink from water pipettes with minimal weight loss. Mean total drinking was recorded on the 3rd
189 day of water restriction. Novel taste consumption groups were presented with 1.0mL of either 0.5%
190 saccharin (*Saccharin 1x*), or Quinine 0.014% (*Quinine 1x*). One hour following the final taste
191 presentation, animals were subjected to patch-clamp electrophysiology (Kayyal et al., 2021;
192 Yiannakas et al., 2021). The *Water* group underwent the same behavioral procedure without novel
193 taste presentations were sacrificed for electrophysiological investigations one hour following
194 water presentation. To dissociate between taste identity and familiarity-related changes in
195 electrophysiological properties, a cohort of mice treated to label the aIC-BLA projection were
196 similarly water deprived following familiarization with saccharin (*Saccharin 5x*). Following the
197 initial water restriction, *Saccharin 5x* animals were allowed access to 0.5% saccharin, in 20minute
198 sessions for 4 days. On the fifth day, mice were provided with 1.0ml of the tastant, 1 hour prior to
199 sacrifice for electrophysiological recordings. Additionally, WT animals injected with the same
200 viral vector, were allowed a month to recover, following which they were sacrificed for
201 electrophysiological investigations without any behavioral manipulation (*Cage Controls*).

202

203 [Electrophysiological studies of the influence of learned aversive taste memory retrieval on](#) 204 [aIC-BLA excitability](#)

205 WT mice were treated with viral constructs labelling aIC-BLA projecting neurons to assess the
206 electrophysiological properties of the projection during aversive or appetitive taste memory
207 retrieval. Upon recovery, mice in CTA retrieval group were trained in CTA for saccharin (LiCl

208 0.14M, 1.5% body weight), while the appetitive saccharin retrieval group (*Saccharin 2x*) received
209 a matching body weight adjusted injection of saline (Yiannakas et al., 2021). Three days following
210 conditioning, both groups underwent a memory retrieval task, receiving 1.0mL of the conditioned
211 tastant 1 hour prior to sacrifice (Figures 2, 4). Brain tissue was extracted and prepared for
212 electrophysiological recording, as above.

213

214 Electrophysiological studies of the influence of learned aversive taste memory extinction 215 and reinstatement on aIC-BLA excitability

216 Electrophysiological studies of CTA extinction and reinstatement were conducted in a cohort of
217 WT male mice (Yiannakas et al., 2021). Following surgery, recovery and water restriction, animals
218 were randomly assigned to the extinction and reinstatement groups (Figures 3-4). The aversion
219 index for the extinction and reinstatement groups were calculated by the formula.

$$220 \text{ Aversion index} = \left[\frac{\text{Volume of water}}{\text{volume of (water+tastant)}} \right] * 100.$$

221 Adult male mice used to study extinction and reinstatement were trained in CTA for saccharin
222 following extinction, the reinstatement group received an identical intraperitoneal dose to the
223 original unconditioned stimulus (LiCl 0.14M, 1.5% body weight), 24 hours prior to retrieval.
224 Conversely, the extinction group received a similarly weight-adjusted dose of saline. During the
225 final retrieval session, both groups of mice were allowed access to 1.0mL of the CS, 1 hour prior
226 to sacrifice under deep anesthesia and slice preparation for electrophysiology.

227

228 Electrophysiology tissue preparation

229 The slice electrophysiology and recording parameters were used as described previously (Kayyal
230 et al., 2021; Yiannakas et al., 2021). Briefly, mice were deeply anesthetized using isoflurane, while
231 brains were extracted following decapitation. Three-hundred um thick coronal brain slices were
232 obtained with a Campden-1000® Vibratome. Slices were cut in ice-cold sucrose-based cutting
233 solution containing the following (in mM): 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH₂PO₄, 28
234 NaHCO₃, 0.5 CaCl₂, 7 MgCl₂, 5 D-glucose, and 0.6 ascorbate. The slices were allowed to recover
235 for 30 min at 37°C in artificial CSF (ACSF) containing the following (in mM): 125 NaCl, 2.5 KCl,
236 1.25 NaH₂PO₄, 25 NaHCO₃, 25 D-glucose, 2 CaCl₂, and 1 MgCl₂. Slices were then kept for an
237 additional 30 min in ACSF at room temperature until electrophysiological recording. The solutions
238 were constantly gassed with carbogen (95% O₂, 5% CO₂).

239

240 Intracellular whole-cell recording

241 After the recovery period, slices were placed in the recording chamber and maintained at 32-34°C
242 with continuous perfusion of carbogenated ACSF (2 ml/min). Brain slices containing the anterior
243 insular cortices were illuminated with infrared light and pyramidal cells were visualized under a
244 differential interference contrast microscope with 10X or 40X water-immersion objectives
245 mounted on a fixed-stage microscope (BX51-WI; Olympus®). The image was displayed on a
246 video monitor using a charge-coupled device (CCD) camera (QImaging®, Canada). Insula to BLA
247 projection cells infected with AAV were identified by visualizing mCherry⁺ cells. Recordings were
248 amplified by Multiclamp™ Axopatch™ 200B amplifiers and digitized with Digidata® 1440
249 (Molecular Devices®). The recording electrode was pulled from a borosilicate glass pipette (3–5
250 M) using an electrode puller (P-1000; Sutter Instruments®) and filled with a K-gluconate-based

251 internal solution containing the following (in mM): 130 K-gluconate, 5 KCl, 10 HEPES, 2.5
252 MgCl₂, 0.6 EGTA, 4 Mg-ATP, 0.4 Na₃GTP and 10 phosphocreatine (Na salt). The osmolarity was
253 290 mOsm, and pH was 7.3. The recording glass pipettes were patched onto the soma region of
254 mCherry⁺ pyramidal neurons and neighboring non fluorescent pyramidal neurons.

255 The recordings were made from the soma of insula pyramidal cells, particularly from layer 2/3 and
256 Layer 5/6. Liquid junction potential (10 mV) was not corrected online. All current clamp
257 recordings were low pass filtered at 10 kHz and sampled at 50 kHz. Pipette capacitance and series
258 resistance were compensated and only cells with series resistance smaller than 20 MΩ were
259 included in the dataset. Data quantification was done with Clampfit (Molecular Devices,
260 Sunnyvale, CA) and subsequently analyzed using GraphPad Prism®. The method for measuring
261 active intrinsic properties was based on a modified version of previous protocols (Kaphzan et al.,
262 2013; Chakraborty et al., 2017; Sharma et al., 2018).

263

264 Recording parameters

265 Resting membrane potential (RMP) was measured 10 sec after the beginning of whole-cell
266 recording (rupture of the membrane under the recording pipette). The dependence of firing rate on
267 the injected current was obtained by injection of current steps (of 500ms duration from 0 to 400
268 pA in 50 pA increments). Input resistance was calculated from the voltage response to a
269 hyperpolarizing current pulse (-150 pA). SAG ratio was calculated from voltage response -150
270 pA. The SAG ratio during the hyperpolarizing steps was calculated as $[(1-\Delta V_{ss}/\Delta V_{max}) \times 100\%]$
271 as previously reported by (Song, Ehlers, & Moyer, 2015). The membrane time constant was
272 determined using a single exponential fit in the first 100ms of the raising phase of cell response to
273 a 1 second, -150 pA hyperpolarization step.

274 For measurements of a single action potential (AP), after initial assessment of the current required
275 to induce an AP at 15ms from the start of the current injection with large steps (50 pA), a series of
276 brief depolarizing currents were injected for 10ms in steps of 10 pA increments. The first AP that
277 appeared on the 5ms time point was analyzed. A curve of dV/dt was created for that trace and the
278 30 V/s point in the rising slope of the AP was considered as threshold (Chakraborty et al., 2017).
279 AP amplitude was measured from the equipotential point of the threshold to the spike peak,
280 whereas AP duration was measured at the point of half-amplitude of the spike. The medium after-
281 hyperpolarization (mAHP) was measured using prolonged (3 seconds), high-amplitude (3 nA)
282 somatic current injections to initiate time-locked AP trains of 50 Hz frequency and duration (10 –
283 50 Hz, 1 or 3 s) in pyramidal cells. These AP trains generated prolonged (20 s) AHPs, the
284 amplitudes and integrals of which increased with the number of APs in the spike train. AHP was
285 measured from the equipotential point of the threshold to the anti-peak of the same spike (Gulledge
286 et al., 2013). Fast (fAHP), and slow AHP (sAHP) measurements were identified as previously
287 described (Andrade et al., 2012; Song and Moyer, 2018). Series resistance, R_{in} , and membrane
288 capacitance were monitored during the entire experiment. Changes of at least 30% in these
289 parameters were criteria for exclusion of data.

290 **Classification of Burst and Regular spiking neurons**

291 At the end of recordings, neurons were classified as either burst (BS) or regular spiking (RS) as
292 reported previously (Kim et al., 2015; Song et al., 2015). Briefly, neurons that fired two or more
293 action potentials (doublets or triplets) potential towards a depolarizing current step above the spike
294 threshold current were defined as burst spiking (BS). Regular spiking (RS) neurons on the other
295 hand, were defined as neurons that fired single action potential in response to a depolarizing current
296 step above spike threshold (Extended Figure 1-2A).

297

298 **Statistical analysis of individual intrinsic properties across treatments**

299 Group size was based on previously published results using similar methods (Gould et al., 2021;
300 Kayyal et al., 2021), as well as through conducting power analysis calculations, in order to obtain
301 power ≥ 0.8 with $\alpha=0.05$ (<https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html>). Individual
302 intrinsic properties of aIC-BLA projecting neurons in the respective treatment groups (Figures 1-
303 4) were analyzed using appropriate statistical tests (One-way or Two-way ANOVA, GraphPad
304 Prism®), as defined in the Table 5: Statistics table. Two-way repeated measurements of analysis
305 of variance (RM-ANOVA) followed by Sidak's (for two groups) or Tukey's (for more than two
306 groups) post-hoc multiple comparison test was performed for firing properties. The intrinsic
307 properties were determined with Two-tailed unpaired t-tests, and One-way ANOVA followed by
308 Tukey's or Dunn's multiple comparisons test were used. For all tests, $*p < 0.05$ was considered
309 significant. D'Agostino & Pearson test used for the identifying the normal distribution of the data.
310 Multiple comparisons were corrected post hoc with Tukey's for One-way/ Two-way ANOVA and
311 Dunn's for Kruskal-Wallis test.

312 Following spike-sorting, the ratio of BS:RS aIC-BLA projecting neurons in the sampled population
313 was compared across our treatments (Mann-Whitney test, GraphPad Prism®). Similarly,
314 individual intrinsic properties in BS and RS aIC-BLA projecting neurons were analyzed following
315 spike-sorting (One-way or Two-way ANOVA, GraphPad Prism®). All data reported as mean \pm
316 standard error (SEM).

317

318 Immunohistochemistry

319 From each electrophysiological recording, three 300 μ m-thick mouse brain slices were obtained
320 starting from Bregma coordinates 1.78, 1.54 and 1.18, respectively. Slices were washed with PBS
321 and fixed using 4% paraformaldehyde in PBS at 4⁰C for 24 hours. Slices were then transferred to
322 30% sucrose/PBS solution for 48 hours and mounted on glass slides using Vectashield[®] mounting
323 medium with DAPI (H-1200). Slides were then visualized using a vertical light microscope at 10x
324 and 20x magnification (Olympus CellSens Dimension[®]). Images were processed using Image-Pro
325 Plus[®] V-7 (Media Cybernetics). The localization of labelled mCherry+ neurons in the agranular
326 aIC - where recordings were obtained from, was quantified manually across three Bregma-matched
327 slices, for each animal. Quantification was done using randomly assigned IDs for individual
328 animals, regardless of treatment. Representative images were additionally processed using the
329 Olympus CellSens 2-D deconvolution[®] function.

330

331 Principal component analysis (PCA) of the profile of intrinsic properties across treatment 332 groups

333 Principal component analysis (PCA) of the standardized intrinsic properties of the LIV-VI aIC-
334 BLA (Figure 5; Extended Figure 5-1) was performed using the correlation matrix on GraphPad
335 Prism9, MATLAB R2020b, and IBM SPSS Statistics 27. The covariance matrix was used for each
336 PCA was performed in six behavioral groups, the low memory prediction (Saccharin 1x, n=20;
337 Saccharin 2x, n=20, and Extinction, n=14), and the high memory prediction (Saccharin 5x, n=18;
338 CTA retrieval, n=27, and Reinstatement, n=15), RS vs. BS neurons. A total of 114 neurons (BS vs.
339 RS) across all intrinsic properties and excitability changes (50–400 pA) (Extended Figure 5-1A),
340 and later all intrinsic properties with only 350 pA (highest excitability differences between

341 treatment groups; Extended Figure 5-1, B). PCA was conducted on 63 burst spiking neurons using
342 12 variables: 350 (pA), RMP (mV), mAHP (mV), sAHP (mV), fAHP (mV), IR ($M\Omega$), Sag Ratio,
343 Time constants (ms), AP amplitude (mV), AP Halfwidth (ms), AP threshold (mV), Rheobase (pA),
344 (Figure 5 A &B). The adequacy of the sample was evaluated using the Bartlett's test and the Kaiser-
345 Meyer-Olkin (KMO) measure was applied. The degrees of freedom (df) were calculated using the
346 following formula:

$$347 \quad df = \#variables - 1.$$

348 The number of principal components was chosen according to the percentage of variance explained
349 (>75%). The parallel analysis evaluated the optimal number of components and selected 3 PCs,
350 explaining 62.47% of the variance. Oblique factor rotation (par) of the first three PCA components,
351 using a standard 'rotatefactors' routine from MATLAB Statistics Toolbox. This approach
352 maximizes the varimax criterion using an orthogonal rotation. To optimize variance, oblique factor
353 rotation (paramax) was used, and the threshold chosen to define a variable as a significant
354 contributor was a variance ≥ 0.7 given the small sample size. The correlation matrix was adequate
355 as the null hypothesis of all zero correlation was rejected [$\chi^2_{66}=387.444$, $p<0.001$], and KMO
356 exceeded 0.5 (KMO=0.580).

357 To calculate the proportion of the variance of each variable that the principal components can
358 explain, communalities were calculated and ranged from 0.426 to 0.897 (extended Figure 5-1, A-
359 C). The communalities scores were calculated using the following formula: $= \frac{\sum_{i=1}^m \lambda}{\#variables * \lambda}$; where
360 m is the number of selected PCs. The threshold chosen was $Comm \geq 60\%$.

361 Due to the imbalance in sample sizes between groups, the PCA space is biased in favor of the
362 group with bigger sample size. The BS neurons in the six behavioral groups previously mentioned
363 were resampled to ensure that sample sizes were balanced across groups datasets (Figure 5, A&B).

364 Particularly, we reduced the number of Saccharin 1X and 2X observations by using random sam-
365 pling ("randn" function in MATLAB); for Saccharin 1x, we chose 10 of the 17 total elements, and
366 for Saccharin 2x, we selected 10 of the 13 total elements.

367

368 K-means clustering

369 Unbiased clustering analysis based on the K-means algorithm was conducted to search for an op-
370 timal division of samples into a pre-determined number of clusters within an unlabeled multidimensional data set (MacQueen, 1967). In the present study we chose K-means clustering methods
371 to cluster the similar groups into a high and low predictive valance outcome following the memory,
372 based on their intrinsic properties. As a popular method for a cluster analysis, K-means clustering
373 aims to partition n observations into k –clusters in which each observation belongs to the cluster
374 with the nearest mean, serving as a prototype of a cluster. In this classification a set of clustering
375 results with different number of clusters can be calculated by setting a different k. The final step
376 is to determine the optimal dimension which gives the best classification. To assess the distribution
377 of bursting neurons in multidimensional space, we performed a k-means cluster analysis in
378 MATLAB for the principal components (k = 2 clusters, maximum iterations was 100 with random
379 starting locations, squared Euclidean distance metric used), which explained 62.47% of the vari-
380 ance in intrinsic properties (Figure 5; extended Figure 5-1 and 5-2).

382

383 Results

384 To prove or refute our hypotheses, we conducted a series of electrophysiological recordings in
385 slice preparation from the mouse aIC, in which we labelled aIC-BLA projecting neurons using
386 retrograde adeno-associated viral tracing – retro AAV (see Methods). Electrophysiological

387 recordings were obtained from the aIC-BLA projecting neurons in LI-III (Table 2) and LIV-VI
388 (Table 1), following novel appetitive or aversive taste stimuli (Figure 1), following appetitive or
389 aversive taste memory retrieval (Figure 2), as well as following extinction and reinstatement
390 (Figures 3-4). We focused on deep-lying LIV-VI aIC-BLA projecting neurons (Table 1), as
391 intrinsic properties in superficial LI-III aIC-BLA projecting neurons were unaffected by taste
392 identity, familiarity, or valence (Table 2). We measured action potential (AP) firing frequency in
393 response to incrementally increasing depolarizing current injections, as well as 11 distinct of
394 intrinsic properties (Tables 1-4 and Table 5: Statistics Table): resting membrane potential (RMP);
395 slow, medium and fast after-hyperpolarization (sAHP, mAHP, fAHP); input resistance (IR), SAG
396 ratio; the amplitude, half-width and threshold for APs; the time taken for a change in potential to
397 reach 63% of its final value (membrane time constant - τ), as well as the minimum current
398 necessary for AP generation (Rheobase). Statistical analysis was conducted using repeated
399 measures one- or two-way ANOVA (see Table 5: Statistics table).

400

401 **When taste is both appetitive and novel, excitability in LIV-VI aIC-BLA projection neurons**
402 **is increased**

403 To delineate the mechanisms through which novelty is encoded on the LIV/VI aIC-BLA
404 projection, we labeled the projection (see methods), and compared the intrinsic properties across
405 neutral, innately aversive, and innately appetitive taste stimuli. Following surgery recovery mice
406 were randomly assigned to the following behavioral groups: Water (Control for procedure, Water;
407 n=6 animals, 23 cells), 0.5 % Saccharin for the first (Novel innate appetitive, Saccharin 1x; n=5
408 animals, 20 cells), or fifth time (Familiar appetitive, Saccharin 5x; n=6 animals, 18 cells), 0.04%
409 Quinine (Novel aversive, Quinine 1x; n=4 animals, 19 cells), or cage controls that did not undergo

410 water-restriction (Base-line control, Cage control; n=4 animals, 19 cells). This approach allowed
411 us to examine excitability changes that relate to the innate aversive/appetitive nature and
412 novelty/familiarity associated with tastants, while accounting for the effects of acute drinking, as
413 well as the water restriction regime itself. Guided by evidence regarding the induction of plasticity
414 cascades, the expression of immediate early genes, as well as the timeframes involved in LTP and
415 LTD in IC neurons (Rosenblum et al., 1997; Hanamori et al., 1998; Jones et al., 1999; Escobar and
416 Bermúdez-Rattoni, 2000), the five treatment groups were sacrificed 1 hour following taste
417 consumption. Even though changes in activity can be observed within seconds to minutes,
418 depending on their novelty, salience and valence (Barot et al., 2008; Lavi et al., 2018; Wu et al.,
419 2020), sensory experiences can modulate the function of IC neurons for hours (Juárez-Muñoz et
420 al., 2017; Rodríguez-Durán et al., 2017; Haley et al., 2020; Kayyal et al., 2021; Yiannakas et al.,
421 2021). We had previously identified a CaMKII-dependent short-term memory trace at the IC that
422 last for the first 3 hours following taste experiences, regardless of their valence (Adaikkan and
423 Rosenblum, 2015). To address whether similar time-dependency of the physiological correlations
424 engaged by the IC during novel taste learning, a 6th group was sacrificed 4 hours following novel
425 saccharin exposure (Figure 1 – Saccharin 1x (4hrs)).

426 Daily water intake prior to the final taste exposure and was not different among the five groups
427 that underwent water restriction (Figure 1B, One-way ANOVA, $p=0.4424$, $F=0.9766$, R squared
428 $=0.1634$). However, excitability in response to incremental depolarizing currents was significantly
429 different between the six groups (Figure 1D – Two-way ANOVA, $p<0.0001$, $F(8,880)=1269$).
430 Exposure to saccharin for the first time (i.e., novel appetitive), at the 1-hour time-point, resulted
431 in enhanced excitability on the aIC-BLA projection compared to all other groups (Figure 1D, see
432 Table 1). Conversely, fAHP (Figure 1H; One-way ANOVA, $p<0.0001$, $F=8.380$, R squared

433 =0.2758) in the Quinine 1x group was increased compared to all other groups, in contrast to
434 Saccharin 1x where it was most decreased (see Table 1). In fact, fAHP in the Saccharin groups
435 recorded at 1hr ($p<0.0001$, $z =5.150$) or 4hours ($p=0.0099$, $z =3.406$) following novel taste
436 consumption was decreased compared to innately aversive Quinine 1x (Figure 1H). Even though
437 fAHP in the Saccharin 1x group was decreased compared to both the Cage control ($p=0.0136$, z
438 $=3.318$) and Water ($p=0.0177$, $z =3.243$) groups, this was not the case for the Saccharin 1x (4hr)
439 group ($p>0.9999$ for both – see Table 1). Importantly, fAHP (Figure 1H) was nearly identical in
440 treatment groups where the tastant could be deemed as highly familiar and safe, such as the Cage
441 control group (that did not undergo water restriction), as well as animals in the Water or Saccharin
442 5x groups (that had undergone water restriction).

443 Significant differences in terms of τ (Figure 1J; One-way ANOVA, $p <0.0001$, $F (5, 110)$; R
444 squared, 0.2169), were observed between the Cage control and Saccharin 1x (4hrs) groups
445 ($p=0.0003$, $q= 6.326$), Water vs. Saccharin 1x (4hr) ($p = 0.0488$, $q= 4.115$), Saccharin 1x and
446 Saccharin 1x (4hrs) groups ($p=0.0002$, $q= 6.521$), and Saccharin 5x vs. Saccharin 1x (4hr), $p =$
447 (0.0081 , $q= 4.975$, $df=110$). On the other hand, significant differences in AP half-width (Figure
448 1I; Kruskal-Wallis test; $p=0.0125$, Kruskal-Wallis statistic=14.54) were only observed between
449 the Saccharin 1x (4hrs) compared to Saccharin 1x ($p=0.0065$, $z =3.519$) groups (see Table 1).

450 These results demonstrate that in the context of taste novelty, innately appetitive saccharin drove
451 increases in excitability and decreases in fAHP of LIV-VI aIC-BLA projecting neurons, compared
452 to innately aversive quinine (Figure 1D, H). Compared to the Cage control and Water groups,
453 fAHP on the projection was significantly enhanced by innately aversive quinine and was decreased
454 by innately appetitive novel saccharin (Figure 1H). However, the effect of appetitive taste novelty
455 on firing frequency was time-dependent, as it was observed at 1hr, but not 4hrs following novel

456 taste exposure (Figure 1D). Furthermore, following familiarity acquisition for saccharin
457 (Saccharin 5x), excitability was decreased compared to Saccharin 1x, matching the Cage control,
458 Water, and Quinine 1x groups (Figure 1D). This led us to consider whether increased excitability
459 is not related to taste identity or palatability (Wang et al., 2018), but the perceived salience of taste
460 experiences, which encompasses both novelty and valence (Ventura et al., 2007; Kargl et al.,
461 2020). Previous studies have suggested that the induction of plasticity signaling cascades and IEGs
462 in pyramidal neurons of the aIC (commonly used as surrogates for changes in excitability), is a
463 crucial step for the association of taste and visceral information during CTA learning (Adaikkan
464 and Rosenblum, 2015; Soto et al., 2017; Wu et al., 2020). Activation of the aIC-BLA projection
465 is indeed necessary for the expression of neophobia towards saccharin (Kayyal et al., 2021), as
466 well as for CTA learning and retrieval (Kayyal et al., 2019). Yet, its chemogenetic inhibition does
467 not affect the attenuation of neophobia, nor the expression of aversion towards innately aversive
468 quinine (Kayyal et al., 2019). Furthermore, aversive taste memory retrieval necessitates increases
469 in pre-synaptic inhibitory input on the projection (Yiannakas et al., 2021). Bearing this in mind,
470 we hypothesized that increases in excitability on the projection could be indicative of a labile state
471 of the taste trace at the aIC, which manifests when taste cues are not (yet) highly predictive of the
472 visceral outcome of the sensory experience (Bekisz et al., 2010; Galliano et al., 2021). In such a
473 scenario, taste memory retrieval following strong single-trial aversive learning would be expected
474 to result in decreased excitability compared to control animals. To assess this hypothesis, we next
475 examined intrinsic excitability in mice retrieving an appetitive (Saccharin 2x, CTA retrieval
476 control) or learned aversive memory (CTA retrieval) for saccharin.

477

478 Learned aversive taste memory retrieval decreases the excitability of LIV-VI aIC-BLA
479 projecting neurons

480 Following recovery from rAAV injection, mice in the CTA retrieval group underwent water
481 restriction and CTA conditioning for 0.5% saccharin (see Methods, Figure 2A).
482 Electrophysiological recordings were obtained from aIC-BLA neurons 3 days later, 1 hour
483 following retrieval (n=8 animals, 27 cells). Mice in the Saccharin 2x group on the other hand, were
484 familiarized with saccharin without conditioning, and recordings were obtained within the same
485 period, following retrieval (n=5 animals, 20 cells). Through this approach we aimed to examine
486 the hypothesis that like innately aversive and highly familiar appetitive responses (Figure 1),
487 learned aversive taste memory retrieval would be correlated with suppression of the intrinsic
488 excitability on the projection.

489 As expected, CTA retrieval mice, exhibited decreased consumption of the conditioned tastant
490 compared to control animals that were only familiarized with saccharin (Figure 2B, Mann-Whitney
491 test, $p=0.0085$; Sum of ranks: 52.50, 38.50; Mann-Whitney $U=2.500$). Intrinsic excitability in
492 LIV-VI aIC-BLA projecting neurons was increased in response to depolarizing current injections
493 (Figure 2D; $p<0.0001$, $F(8, 360)=483.3$), and was significantly different between the two
494 treatments ($p=0.0014$, $F(1, 45)=11.60$). Excitability was enhanced in the Saccharin 2x group
495 compared to CTA retrieval, while a significant interaction was identified between the treatment
496 and current injection factors ($p<0.0001$, $F(8, 360)=9.398$). Fast AHP (fAHP) on LIV-VI aIC-BLA
497 projecting neurons tended to be increased in the CTA retrieval group (see Table 1), however
498 differences compared to Saccharin 2x failed to reach significance (Unpaired t-test; $p=0.0527$,
499 $t=1.990$, $df=45$). Conversely, AP amplitude in the Saccharin 2x group was significantly decreased
500 compared to CTA retrieval (Figure 2G; Unpaired t-test; $p=0.0002$, $t=3.983$, $df=45$). In addition,

501 the CTA retrieval group exhibited significantly decreased IR (Figure 2H; Unpaired t-test;
502 $p=0.0036$, $t=3.072$, $df=45$) and significantly enhanced SAG ratio (Figure 2I; Unpaired t-test;
503 $p=0.0037$, $t=3.060$, $df=45$), compared to Saccharin 2x. In accord with our hypothesis, excitability
504 on LIV-VI aIC-BLA projecting neurons was decreased by aversive taste memory retrieval. We
505 have previously shown that compared to CTA retrieval and reinstatement, appetitive memory
506 retrieval and extinction were associated with (a) an enhancement of IEG induction (c-fos and
507 Npas4) at the aIC, and (b) decreased frequency of pre-synaptic inhibition on the aIC-BLA
508 (Yiannakas et al., 2021). In accord, other published work investigating the induction of IEG in the
509 rodent IC, found that consistent with a reduction in spiking activity (Grossman et al., 2008), the
510 induction of c-fos in IC neurons was decreased by aversive taste memory retrieval (Haley et al.,
511 2020). Earlier studies have also reported increases in c-fos following the extinction of cyclosporine
512 A-induced CTA (Hadamitzky et al., 2015). We thus hypothesized that if excitability in these cells
513 serves as key node for a change in valence prediction, extinction - which constitutes a form of
514 appetitive re-learning, would be associated with enhanced excitability compared to CTA retrieval
515 and reinstatement (Berman, 2003; Suzuki et al., 2004; Morrison et al., 2016; Slouzkey and
516 Maroun, 2016). In addition, through these extinction and reinstatement studies, we were able to
517 examine the real-life relevance of these changes on intrinsic excitability, in a context where
518 behavioral performance reflects the balance between contrasting memories and the availability of
519 retrieval cues (Figure 3).

520

521 The predictability of the valence arising from taste experiences determines the profile of
522 intrinsic properties of LIV-VI aIC-BLA projecting neurons

523 Using similar approaches, electrophysiological recordings were obtained from LIV-VI aIC-BLA

524 projecting neurons from mice having undergone unreinforced CTA extinction (Extinction; n=5, 14
525 cells), or US-mediated CTA reinstatement (Reinstatement; n=3 animals, 15 cells). Behaviorally,
526 the two groups of animals were similar in terms of their aversion profile over 9 unreinforced
527 extinction sessions (Figure 3B; 2-way ANOVA; Extinction: $p < 0.0001$, $F(8, 54) = 13.44$;
528 Treatment: $p = 0.0681$, $F(1, 54) = 3.466$; Interaction: $p = 0.9697$, $F(8, 54) = 0.2803$). As expected,
529 saccharin consumption during the test day in the Reinstatement group was decreased compared to
530 Extinction (Figure 3C; Mann-Whitney test; $p = 0.0179$; Sum of ranks: 30, 6; Mann-Whitney $U = 0$).
531 Consistent with our findings in Figure 2, aversive taste memory retrieval in the Reinstatement
532 group was associated with decreased excitability compared to the Extinction group (Figure 3E; 2-
533 way ANOVA, Current injection: $p < 0.0001$, $F(8, 216) = 370.1$; Treatment: $p = 0.0297$, $F(1, 27)$
534 $= 5.291$; Interaction: $p < 0.0001$, $F(8, 216) = 10.30$). CTA Reinstatement was also associated with
535 increases in the AP threshold (Figure 3F; Unpaired t-test: $p = 0.0076$, $t = 2.887$, $df = 27$) and τ (Figure
536 3G; Unpaired t-test: $p = 0.0153$, $t = 2.589$, $df = 27$) compared to Extinction.

537 Unlike animals that underwent familiarization with the tastant without conditioning (Figure 1),
538 excitability on the projection in the Extinction group was not decreased by familiarization (Figure
539 3). Conversely, even though the intrinsic mechanisms employed would appear to differ, aversive
540 taste memory retrieval regardless of prior experience, was associated with baseline excitability of
541 the aIC-BLA projection (Figure 3). Our findings in this section (Figure 3), revealed that during
542 taste memory retrieval, excitability on the projection is not solely dependent on the relevant
543 novelty or appetitive nature of tastants, and does not subservise the persistence of CTA memories
544 (Figure 2). Instead, excitability on the aIC-BLA projection is indeed shaped by prior experience
545 but is best predicted by the probability for further aversive (re)learning.

546 Next, to distinguish between intrinsic properties changes that reproducibly reflect taste identity,

547 familiarity, and valence over the course of time and experience, we compared the profile of
548 intrinsic properties across pairs of behavioral groups in which the currently perceived novelty, as
549 well as innate or learned valence associated with taste was notably different. Through this
550 comparison we were led to conclude that excitability on aIC-BLA projecting neurons is driven by
551 taste stimuli of positive valence, however this effect is dependent on subjective experience and the
552 possibility for further associative learning (Figure 4A). Excitability on aIC-BLA projecting
553 neurons in the treatment groups where the tastant was perceived as appetitive (Saccharin 1x,
554 Saccharin 2x and Extinction), was closely matched, and was significantly enhanced compared to
555 the innately or learned aversive (Quinine 1x, CTA retrieval and Reinstatement) groups (Figure 4A;
556 Two-way ANOVA; Current injection: $p < 0.0001$, $F(5, 872) = 1218$; Treatment: $p = 0.0014$, $F(5,$
557 $109) = 4.281$; Interaction: $p < 0.0001$, $F(40, 872) = 4.978$). As previously identified in Figure 1H,
558 fAHP reflected the innate aversive nature of the tastant, being increased in the Quinine 1x group
559 compared to all other groups (Figure 4B; One-way ANOVA; $F = 10.65$, $p < 0.0001$, R squared
560 $= 0.3283$, see Table 1). Significant differences in IR (Figure 4C; One-way ANOVA; $F = 2.775$,
561 $p = 0.0213$, R squared $= 0.1129$) and SAG ratio (Figure 4D; One-way ANOVA; $F = 2.610$, $p = 0.0286$,
562 R squared $= 0.1069$) were only observed between the CTA retrieval and Saccharin 2x groups. AP
563 amplitude (Figure 4E; One-way ANOVA, $p = 0.0054$, $F = 3.526$, R squared $= 0.1392$) in the
564 Saccharin 2x group was decreased compared to both CTA retrieval ($p = 0.0129$, $q = 4.768$, $df = 109$)
565 and Quinine 1x ($p = 0.0087$, $q = 4.944$, $df = 109$). Conversely, the Extinction and Reinstatement
566 groups, where familiarity with the tastant was the highest, exhibited increased AP half-width
567 compared to all other groups (Figure 4F; One-way ANOVA, Kruskal-Wallis test; $p = 0.0002$;
568 Kruskal-Wallis statistic, 24.03). Significant differences in terms of τ (Figure 4G; One-way
569 ANOVA, $p = 0.0047$, $F = 3.606$) were only observed in comparing the Saccharin 1x and

570 Reinstatement groups ($p=0.0022$, $q=5.525$, $df=109$). Hence, neuronal excitability is indeed a
571 feature associated with predictive power to modulate taste valence, however it does not fully reflect
572 the breadth of intrinsic property changes among the different behavioral groups.

573

574 The predictability of taste valence intrinsic is primarily reflected on the excitability of burst
575 spiking, but not regular spiking LIV-VI aIC-BLA projecting neurons

576 Our initial analysis of individual intrinsic properties (Figures 1-3) highlighted that excitability is
577 enhanced following appetitive experiences in which the internal representation is still labile and is
578 associated with the possibility for further aversive learning (novelty or extinction). Conversely,
579 following extensive familiarization, aversive conditioning, or reinstatement, whereby taste
580 exposure leads to memory retrieval of specific valence, excitability on LIV-VI aIC-BLA projecting
581 neurons was similar to baseline (Figures 1 and 4). While the precise mechanism through which
582 sensory input is encoded at the cortex (and other key regions), is still a matter of ongoing research,
583 studies indicate that bursting in cortical layer V pyramidal neurons can encode oscillating currents
584 into a pattern that can be reliably transmitted to distant post-synaptic terminals (Kepecs and
585 Lisman, 2003; Samengo et al., 2013; Zeldenrust et al., 2018). Spike burst is defined as the
586 occurrence of three or more spikes from a single neurons with $<8\text{ms}$ intervals (Ranck, 1973;
587 Connors et al., 1982). In brain slices from naïve mice, half of the neurons of a given structure
588 exhibit burst firing, while the distribution of burst spiking (BS) to regular spiking (RS) neurons,
589 changes along the anterior-posterior axis of the subiculum (Staff et al., 2000; Jarsky et al., 2008).
590 Importantly, the two cell types fine-tune the output of brain structures by virtue of differences in
591 synaptic plasticity, as well as intrinsic excitability mechanisms (Graves et al., 2012; Song et al.,
592 2012). Furthermore, there are changes in the ratio of BS:RS neurons in individual brain structures,

593 as well as differences in the recruitment of signaling events, ion channels and metabotropic
594 receptors among the two cell types (Wozny et al., 2008; Shor et al., 2009). Correspondingly,
595 complex region and task-specific rules govern the molecular and electrophysiological mechanisms
596 through which information encoding and retrieval takes place in the two cell types (Dunn et al.,
597 2018; Dunn and Kaczorowski, 2019). Little is currently known regarding the influence of cell
598 identity in the repertoire of plasticity mechanisms employed by the IC to facilitate taste-guided
599 behaviors (Maffei et al., 2012; Haley and Maffei, 2018).

600 Our post-hoc spike sorting analysis allowed us to distinguish between BS and RS LIV-VI aIC-
601 BLA projecting neurons, and thus their relative contribution to behaviorally driven changes in the
602 suit of intrinsic properties (Extended Figures 1-3, 2-1 and 3-1). Through this comparison, we
603 uncovered that Saccharin 1x differed to other groups in terms of excitability and fAHP in BS LIV-
604 VI aIC-BLA neurons (Extended Figure 1-3), while no such changes were observed in RS neurons
605 (see Summary of RS intrinsic properties table no.4). Similarly, excitability in the Saccharin 2x
606 group was significantly enhanced compared to CTA retrieval in BS-, but not in RS LIV-VI aIC-
607 BLA neurons (Extended Figure 2-1A, F). Significant differences in IR, SAG ratio and AP
608 amplitude between CTA retrieval and Saccharin 2x were primarily driven by BS LIV-VI aIC-BLA
609 neurons (Extended Figure 2-1B-D, G-I). Conversely, significant differences in AP half-width
610 between the aversive and appetitive memory retrieval groups were only observed in RS neurons
611 (Extended Figure 2-1J). Correspondingly, excitability in the Extinction group was enhanced
612 compared to Reinstatement in BS-, and not RS LIV-VI aIC-BLA neurons (Extended Figure 3-1A,
613 H). Indeed, excitability on BS LIV-VI aIC-BLA neurons following extinction and reinstatement,
614 reflected the subjective predictability of taste memory retrieval, being high following extinction
615 compared to reinstatement (Extended Figure 3-1). However, this effect was mediated through

616 alternative mechanisms compared to single-trial learning and memory retrieval (Extended Figure
617 1-3, 2-1, 3-1). Significant differences between the Extinction and Reinstatement groups, were
618 observed in terms of the sAHP, AP threshold, SAG ratio and τ in BS but not in RS LIV-VI aIC-
619 BLA neurons (Extended Figure 3-1B-F).

620 Encouraged by these findings, we focused on the Saccharin 1x, Saccharin 2x, Saccharin 5x, CTA
621 Retrieval, Extinction and Reinstatement groups, as to isolate the contribution of BS LIV-VI aIC-
622 BLA neurons in encoding the subjective predictability of taste experience during taste learning,
623 re-learning, and memory retrieval (Figure 5). Consistent with studies in the hippocampus (Graves
624 et al., 2016), we found that the percentage of BS LIV-VI aIC-BLA projecting neurons in the
625 sampled population was highest in the context of novel taste learning (Extended Figure 1-2B:
626 Saccharin 1x, 85%), and subsided following progressive familiarization (Extended Figure 1-2B;
627 Saccharin 2x, 65%, Mann-Whitney test: $p=0.0562$; Sum of ranks: 303.5, 161.5; Mann-Whitney U
628 $=70.50$; Saccharin 5x, 55.56%, Mann-Whitney test: $p=0.0034$; Sum of ranks: 291, 87; Mann-
629 Whitney U $=32$). Interestingly, animals retrieving CTA, exhibited the lowest proportion of BS
630 neurons among the six treatments (Extended Figure 1-2B; CTA retrieval, 44.44% BS), and
631 significant differences were observed compared to control animals (Extended Figure 1-2B;
632 Saccharin 2x, 65% BS; Mann-Whitney test: $p=0.0102$; Sum of ranks: 257, 208; Mann-Whitney U
633 $=55$). Thus, the ratio of BS:RS LIV-VI aIC-BLA projecting neurons was plastic in relation to
634 experience and was highest in response to appetitive novelty – in accord with studies investigating
635 the intrinsic excitability of subiculum output neurons in relation to contextual novelty and valence
636 encoding (Dunn et al., 2018). Indeed, the ratio of BS:RS LIV-VI aIC-BLA neurons was
637 progressively decreased by familiarity acquisition (Saccharin 1x > 2x > 5x), as well as following
638 aversive taste memory recall (CTA retrieval), compared to both appetitive learning (Extended

639 Figure 1-2B; Saccharin 1x, Mann-Whitney test: $p < 0.0001$; Sum of ranks: 407, 188; Mann-Whitney
640 $U = 35$) and re-learning (Extended Figure 1-2B; Extinction, Mann-Whitney test: $p = 0.0007$; Sum
641 of ranks: 182, 224; Mann-Whitney $U = 29$). However, our comparison failed to account for the
642 influence of complex experiences over time, as differences between Extinction and Reinstatement
643 failed to reach significance (Extended Figure 1-2B; Mann-Whitney test: $p = 0.3870$, Sum of ranks:
644 133.5, 97.50, Mann-Whitney $U = 42.50$), while perplexingly, the ratio of BS:RS aIC-BLA neurons
645 in these groups was differentially increased compared to Saccharin 5x (Extended Figure 1-2B;
646 Extinction, Mann-Whitney test: $p = 0.0300$; Sum of ranks: 81, 150; Mann-Whitney $U = 26$;
647 Reinstatement, Mann-Whitney test: $p = 0.3498$; Sum of ranks: 90, 120; Mann-Whitney $U = 35$), but
648 not Saccharin 2x (Extended Figure 1-2B; Extinction, Mann-Whitney test: $p = 0.2397$; Sum of ranks:
649 143.5, 156.5; Mann-Whitney $U = 52.50$; Reinstatement, Mann-Whitney test: $p > 0.9999$; Sum of
650 ranks: 153.50, 122.5; Mann-Whitney $U = 62.50$). No further statistical analysis were performed in
651 intrinsic properties of aIC-BLA regular spiking neurons representing (Figure 1 and Figure 3),
652 because of the small sample size.

653 Changes in the intrinsic properties of neuronal ensembles have recently been suggested to
654 contribute to homeostatic mechanisms integrating both cellular and synaptic information (Wu et
655 al., 2021). In our current study, we randomly sampled from neuroanatomically defined LIV-VI
656 aIC-BLA projecting neurons, and even following spike sorting (Extended Figures 1-2), the
657 probability of recording from engram cells (10% of neurons within a region) would be extremely
658 low (Tonegawa et al., 2015). Importantly, the correlative nature does not exclude the possibility
659 that these changes are the consequence of representational drift (Driscoll et al., 2017). We thus set
660 out to examine the hypothesis that applying linear dimension reduction method on the complement
661 of intrinsic properties recorded in BS LIV-VI aIC-BLA neurons would allow us to distinguish

662 between taste experiences that differ in terms of their perceived predictability (or the associated
663 probability for further aversive learning).

664

665 Principal component analysis of the profile of intrinsic properties in BS LIV-VI aIC-BLA
666 projecting neurons separates treatment groups in relation to the perceived predictability of
667 taste valence for saccharin

668 We assigned six of our treatment groups into highly predictive scenarios (Saccharin 5x, CTA
669 retrieval and Reinstatement), and low predictive scenarios (Saccharin 1x, Saccharin 2x,
670 Extinction). We used parallel analysis to select the components across the complement of intrinsic
671 properties in each treatment group, with the first three principal components (PC1-3) explaining
672 30.84%, 17.88%, and 13.75% of the total variance, respectively, and 62.47% of the variance,
673 collectively (Extended Figure 5-2). PC1 (Figure 5B, Extended Figure 5-2) was characterized by
674 strong negative loadings for Rheobase (-0.88304), sAHP (-0.85985) and mAHP (-0.82421), while
675 a positive correlation was identified for IR (0.694764). The direction of PC2 (Figure 5B, Extended
676 Figure 5-2) was positively correlated with fAHP (0.682681) and AP halfwidth (0.614103) and was
677 negatively correlated with Excitability at 350pA (-0.69587). PC3 (Figure 5B, Extended Figure 5-
678 2) positively correlated with SAG ratio (0.889949) and RMP (0.682681), whereas a significant
679 negative correlation with mAHP (-0.6095) was also identified. Unlike aversive or appetitive taste
680 memory retrieval (i.e., highly predictive), appetitive novelty or extinction learning (i.e., low
681 predictive), was associated with increased input resistance, faster action potential generation and
682 decreased afterhyperpolarization on BS aIC-BLA neurons (Figure 5). Importantly, PCA of the
683 intrinsic properties of LIV-VI aIC-BLA projecting neurons regardless of cell type (BS and RS
684 together, Extended Figure 5-1), failed to segregate the two groups of treatments. This cell-type

685 specific profile of intrinsic properties could provide the framework through which BS LIV-VI aIC-
686 BLA projecting neurons are able to inspect the gastrointestinal consequences associated with
687 tastants over prolonged timescales, when these consequences are not accurately predicted by
688 sensory experience or memory retrieval alone (Adaikkan and Rosenblum, 2015; Lavi et al., 2018;
689 Kayyal et al., 2019).

690

691 Discussion

692 Learning and memory are subserved by plasticity in both synapse strength and neuronal intrinsic
693 properties (Citri and Malenka, 2008; Sehgal et al., 2013). While Hebbian rules can explain asso-
694 ciative learning paradigms where seconds separate the CS and US (Krabbe et al., 2018), additional
695 cellular-level mechanisms are needed to explain how learning operates in paradigms where the
696 time between CS and US extends to hours (Adaikkan and Rosenblum, 2015; Wu et al., 2021). In
697 this study, we demonstrate that following taste experiences, taste percept and prior experience are
698 integrated in the intrinsic properties of the aIC in a time-dependent and cell-type specific manner.
699 We further show that regardless of the identity or prior history associated with taste, the intrinsic
700 properties of BS LIV-VI aIC-BLA projecting neurons encodes the perceived confidence of taste
701 valence attribution.

702 We focused on the aIC-BLA projection; a circuit causally involved in the acquisition and retrieval
703 of CTA memories (Kayyal et al., 2019, 2021). We examined the hypothesis that excitability in aIC-
704 BLA neurons can serve as a taste valence updating mechanism enabling the prolonged ISI between
705 CS and US in CTA learning (Adaikkan and Rosenblum, 2015), and/or contributes to anticipatory

706 valence attribution (Barrett and Simmons, 2015). Our basic proposition diverged from Hebb's fa-
707 mous postulate that cells with increased excitability over hours can potentially wire together with
708 cells conveying incoming modified valence information (Hebb, 1961).

709 The confidence with which taste valence is encoded is the product of the subjectively perceived-
710 (a) appetitive or aversive nature of tastants and (b) novelty or familiarity associated with tastants
711 (Russell, 1980; Kahnt and Tobler, 2017). We first examined each axis separately and later in tan-
712 dem as to better simulate real-life scenarios. We measured the intrinsic properties of aIC-BLA
713 neurons 1 hour following taste experience – a previously established suitable time point (Jones et
714 al., 1999; Haley et al., 2020).

715 To dissociate novelty-related changes from those involving hydration, taste identity and familiar-
716 ity; we compared the intrinsic properties of aIC-BLA neurons following Water – a neutral and
717 familiar tastant, Saccharin – an innately appetitive tastant, in the context of novelty (1x) or famil-
718 iarity (5x), and Quinine – an innately aversive novel tastant (Figure 1). Excitability was high fol-
719 lowing novel saccharin exposure, but not in response to Quinine (Figure 1D). Indeed, concerted
720 activity at the aIC and BLA encodes the presence, identity, and palatability of taste experiences
721 within the 2 seconds preceding swallowing (Katz et al., 2001; Grossman et al., 2008; Fontanini et
722 al., 2009). Palatability can be enhanced as a function of experience (Austen et al., 2016), but can
723 also be decreased by sensory satiety and alliesthenia (Rolls et al., 1981; Yeomans, 1998; Siemian
724 et al., 2021). However, excitability on the projection was enhanced in response to novelty and was
725 decreased following familiarization (Figure 1). Further inconsistent with palatability encoding;
726 changes in excitability captured 1 hour following novel saccharin exposure subsided 4 hours later
727 (Figure 1), while excitability remained plastic even following longer periods of water restriction,
728 that could be considered monotonous (Figure 5). Deciphering whether and how aIC-BLA neurons

729 contribute to palatability processing would require *in vivo* recordings to capture taste-evoked
730 changes, within timescales that are beyond the scope and means of our current study (Vincis and
731 Fontanini, 2016).

732 The correlation identified between excitability and innate current taste valence, encouraged us to
733 examine the predictability of future outcomes following aversive taste memory retrieval. Bearing
734 in mind our previous findings using transcription-dependent activity markers at the aIC (Yiannakas
735 et al., 2021), we hypothesized that aversive taste memory retrieval (CTA retrieval or Reinstatement),
736 would be associated with decreased excitability compared to stimulus- and familiarity-
737 matched controls (Saccharin 2x and Extinction). Indeed, excitability on aIC-BLA projecting neu-
738 rons following CTA retrieval was decreased compared to Saccharin 2x (Figure 2B), while Rein-
739 statement, was also associated with decreased excitability compared to Extinction (Figure 3E).
740 Hence, regardless of the complexity of taste memory retrieval, excitability in aIC-BLA neurons
741 was best predicted by the subjective predictability of taste valence - increasing in response to in-
742 nately appetitive taste experiences in which the perceived possibility for avoidance learning was
743 high/taste valence predictability was low (Figure 4). Conversely, when the subjective confidence
744 with which taste valence was predicted was high, excitability on the projection remained un-
745 changed (Figures 1, 4).

746 Innately and learned aversive tastants were both associated with decreased excitability on aIC-
747 BLA projecting neurons compared to appetitive controls, however these effects were mediated
748 through alternative mechanisms (Figures 2-4). Quinine increased fAHP on the projection com-
749 pared to saccharin, regardless of familiarity or perceived valence (Figures 1 and 4). Post-spike
750 after-hyperpolarization (AHP) has a key function in transducing the summed result of processed
751 synaptic input, directly impacting neuronal excitability in relation to both learning and aging (Oh

752 and Disterhoft, 2020). In pyramidal cells of the hippocampus and cortex, differences in fAHP are
753 mediated by the Ca^{2+} and voltage-dependent BK currents that promote repolarization at the be-
754 ginning APs trains (Shao et al., 1999). Interestingly, studies in the prefrontal cortex (PFC), have
755 shown that fear conditioning decreases excitability, whereas extinction training enhances excita-
756 bility and decreases medium- and slow AHP (Santini et al., 2008; Maglio et al., 2021). Chronic
757 ethanol consumption has shown to suppress excitability and to increase AHP in IC neurons (Luo
758 et al., 2021). Conversely, oxytocin-dependent signaling has been shown to promote social affective
759 behaviors, via increases in excitability and decreases in sAHP in IC neurons (Rogers-Carter et al.,
760 2018). Further studies would be necessary to fully address this, but our findings could indicate that
761 enhanced fAHP is induced by innately aversive tastants or quinine specifically.

762 Unlike Quinine, CTA memory retrieval, was associated with increased AP amplitude and SAG
763 ratio, as well as decreased IR in BS LIV-VI aIC-BLA projecting neurons, compared to control
764 animals (Figure 2, Extended Figure 2-1). On the other hand, the decreased excitability in the Re-
765 instatement group compared to Extinction was characterized by decreased AP threshold, increased
766 τ , and decreased sAHP (Figures 3, 4, Extended Figure 3-1). The hyperpolarization-activated, cyclic
767 nucleotide-gated current (I_h) regulates membrane depolarization following hyperpolarization (Ho-
768 gan and Poroli, 2008; Shah, 2014). The opening of HCN channels generates an inward current,
769 that modulates AHP, RMP and IR in cortical pyramidal and PV interneurons (Yang et al., 2018).
770 However, conductance through I_h channels, regulates synaptic integration at the soma of pyramidal
771 neurons, by suppressing excitability, decreasing IR, and increasing τ (Hogan and Poroli, 2008).
772 Evidence indicates that this dichotomous impact of HCN channels on neuronal excitability, is me-
773 diated by A-type K channels at the dendrites (Mishra and Narayanan, 2015), and M-type channels
774 at the soma (Hu et al., 2007). Notably, AP half-width was significantly increased in the Extinction

775 and Reinstatement groups that had undergone extinction training, compared to all other saccharin-
776 treated groups (Figure 4F). Mechanistically, this effect could reflect changes in the distribution
777 and/or the properties of voltage- or calcium-gated ion channels (Faber and Sah, 2002; Grubb and
778 Burrone, 2010; Kuba et al., 2010). Such broadening of spike width has also been reported in in-
779 fralimbic PFC neurons projecting to the amygdala in response to extinction training (Senn et al.,
780 2014). PV-dependent restriction of excitability and burst firing, is instrumental in experience-de-
781 pendent plasticity in the amygdala (Morrison et al., 2016), the hippocampus (Donato et al., 2013;
782 Xia et al., 2017) and visual cortex (Yazaki-Sugiyama et al., 2009; Kuhlman et al., 2013). Con-
783 versely, in the striatum, PV interneurons restrict bursting, calcium influx, and synaptic plasticity
784 to appropriate temporal windows that facilitate learning, but not retrieval (Owen et al., 2018). El-
785 ephant recent studies report that rapid eye movement sleep is associated with a PV-dependent so-
786 matodendritic decoupling in pyramidal neurons of the PFC (Aime et al., 2022). At the IC, the
787 maturation of GABAergic PV circuits is key for multisensory integration and pruning of cross-
788 modal input to coordinate the detection of relevant information (Gogolla et al., 2014). Activation
789 of IC PV disrupts the expression of taste-guided goal-directed behavior (Vincis et al., 2020), and
790 enhances taste-guided aversive responses (Yiannakas et al., 2021). Our findings could be indica-
791 tive of a prediction-dependent decoupling mechanism at the IC, whereby the restriction of bursting
792 activity in LIV-VI aIC-BLA neurons impinges on innate drives towards the tastant and further
793 learning, depending on prior experience.

794 We further probed our results and hypotheses using PCA and attempted to segregate behaviors
795 based on the perceived ability of the CS to predict the consequences of sensory experience, and
796 the probability for further learning (Figure 5). We focused on BS LIV-VI aIC-BLA projecting neu-
797 rons since bursting has been implicated in coincidence detection by deep-layer neurons

798 (Boudewijns et al., 2013; Shai et al., 2015), as well as the encoding of novelty and valence relating
799 to different modalities (Song et al., 2015; Dunn et al., 2018; Yousuf et al., 2019). Our PCA of
800 intrinsic properties in BS LVI-VI aIC-BLA projecting neurons demonstrated that distinct plasticity
801 rules are at play depending on the balance between the probability for associative learning and the
802 certainty with which taste predicts the valence of experience during retrieval (Extended Figure 5-
803 1, 2). We propose that increased excitability and reduced fAHP on BS LIV-VI aIC-BLA projecting
804 neurons might represent a transient neuronal state in the absence of adequate predictive cues for
805 the outcome of taste experiences (Adaikkan and Rosenblum, 2015).

806 The IC has long been considered crucial for interoception, which is increasingly understood to be
807 supported by distinct direct or indirect functional bidirectional connectivity. Indeed, interoceptive
808 inputs relating to the processing, or anticipation of physiological states of hydration and satiety
809 manifest at the pIC (Livneh et al., 2017, 2020; Livneh and Andermann, 2021). However, this is
810 rarely the case when it comes to physiological hydration or satiety inputs and the aIC, that has is
811 primarily involved in interoceptive processes in the context food poisoning or CTA (Chen et al.,
812 2020; Wu et al., 2020). As other studies currently in press demonstrate, hydration correlates and
813 requires decreased activity in aIC-BLA and increases in pIC-BLA CB1 receptor-expressing neu-
814 rons (Zhao et al., 2022). In fact, and in further accord with our previous findings (Lavi et al., 2018;
815 Kayyal et al., 2019), optogenetic activation of the aIC-BLA projection was found to be anxiogenic,
816 while physiological conditions that are associated with negative valence and anxiety were encoded
817 by changes in activity on the projection (Nicolas et al., 2022). Under uncertain conditions that are
818 associated with greater potential significance, recruitment of the aIC is thought to contribute to
819 attention, effort, and accurate processing (Lovero et al., 2009), as to identify better response op-

820 tions (Preuschoff et al., 2008). In agreement with earlier computational models of the cortical con-
821 nectivity (Mumford, 1991, 1992), recent work indicates that the aIC facilitates prediction-related
822 encoding driven by hedonics, rather than homeostatic needs (Darevsky et al., 2019; Price et al.,
823 2019; Darevsky and Hopf, 2020). Our results, propose a cellular framework for such an emotional
824 predictive function at the aIC. Indeed, sex can be an important biological variable when examining
825 brain circuits in relation to behavior (Rogers-Carter et al., 2018). Even though we cannot exclude
826 the possibility of sex-specific differences, in previous studies where we manipulated activity at the
827 IC in a cell-type specific manner, we found no differences between male and female mice (Kayyal
828 et al., 2019; Yiannakas et al., 2021). Future studies will further explore whether and how the inter-
829 play between such distinct mechanisms at the aIC, enables its complex role in learning, memory,
830 and decision-making.

831

832 References

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1158
- 1159

1160 **Figure legends**

1161 Graphical abstract: A model for excitability changes in IV-VI aIC-BLA neurons when the
1162 taste salience is low and highly predictive following the taste memory

- 1163 • The unpredictability of future valence outcomes: Novel appetitive taste experiences /ex-
1164 tinction of previously learned aversive taste experiences, increases the excitability (blue
1165 arrow) of aIC-BLA projecting neurons (red). This increased excitability is specific to the
1166 deep-layer aIC-BLA projecting neurons (LIV-VI).
- 1167 • When the taste valence is highly predictable (familiar/aversive), excitability is reduced in
1168 LIV-VI aIC-BLA projecting neurons (red arrow).
- 1169 • LIV-VI aIC-BLA neurons remain excitable facilitating the association of the taste memory
1170 trace with visceral pain when the stimulus is not adequately predictive of the outcome of
1171 the sensory experience.

1172

1173 **Figure 1: Retrieval of appetitive and novel taste increases excitability in LIV-VI aIC-BLA**
1174 **projection neurons**

1175 A) Diagrammatic representation of experimental procedures. Following surgery and stereotaxic
1176 delivery of ssAAV_retro2-hSyn1-chi-mCherry-WPRE-SV40p(A) into the BLA, mice were
1177 allowed 4 weeks of recovery. Animals were subsequently assigned to treatment groups and trained
1178 to drink from pipettes (see Methods). We compared the intrinsic properties of LIV-VI aIC-BLA
1179 neurons among the Water (n=6 animals, 23 cells), Saccharin 1x (n=5 animals, 20 cells), Saccharin
1180 1x(4h) (n=4 animals, 17 cells), Saccharin 5x (n=6 animals, 18 cells) and Quinine 1x groups (n=4
1181 animals, 19 cells), as well as a Cage control group (n=4 animals, 19 cells) that underwent surgery

1182 and stereotaxic delivery of ssAAV_retro2-hSyn1-chi-mCherry-WPRE-SV40p(A) at the BLA
1183 without water restriction.

1184 B) Graph showing the water consumption prior to treatment (mean \pm SD). There was no significant
1185 difference between water intakes between the groups before the treatment. One-Way ANOVA, $p=$
1186 0.9766.

1187 C) Representative traces of LIV-VI aIC-BLA projecting neurons from the six treatment groups.
1188 Scale bars 20 mV vertical and 50ms horizontal from 300 pA step.

1189 D) The dependence of firing rate on current step magnitude in LIV-VI aIC-BLA neurons was
1190 significantly different among the treatment groups. Excitability in the Saccharin 1x was increased
1191 compared to all other groups. Two-way repeated measures ANOVA, Current x Treatment:
1192 $p<0.0001$; Cage control vs. Saccharin 1x: $**p<0.01$, $***p<0.001$; Saccharin 1x vs. Saccharin 1x
1193 (4hr): $\#p<0.05$, $\#\#p<0.01$, $\#\#\#p<0.0001$; Water vs. Saccharin 1x: $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}$
1194 $p<0.001$; Saccharin 1x vs. Quinine 1x: $\$p<0.05$, $\$\$p<0.01$; Saccharin 1x vs. Saccharin 5x: -
1195 $p<0.05$; Saccharin 1x (4hr) vs. Saccharin 5x: + $p<0.05$.

1196 E) Representative of all fAHP measurements in response to 500 ms step current injections. Scale
1197 bars 20 mV vertical and 50 ms horizontal.

1198 F) Representative of all action potential properties were taken. Scale bars 20 mV vertical and 5 ms
1199 horizontal.

1200 G) Measurements for all input resistance, sag ratio and membrane time constants were analyzed
1201 in response to 1 sec, -150pA step current injection. P- peak voltage, S- steady state voltage. Scale
1202 bars 5 mV vertical and 100 ms horizontal.

1203 H) Significant differences were observed among the treatment groups in terms of fAHP. Cage
1204 control (9.191 ± 1.449 mV), Water (8.150 ± 0.8288 mV), Saccharin 1x (3.016 ± 0.9423 mV),
1205 Quinine 1x (13.58 ± 1.562 mV) Saccharin 5x (8.158 ± 1.356 mV), Saccharin 1x (4hrs) ($5.989 \pm$
1206 1.074 mV), One-Way ANOVA, $p < 0.0001$.

1207 I) Action potential half-width in the Saccharin 1x group (0.6005 ± 0.03260 ms) was significantly
1208 decreased compared to Saccharin 1x (4hr) (0.7765 ± 0.03641 ms), One-Way ANOVA, $p = 0.0065$.

1209 J) The membrane time constant was significantly different between the Cage Control ($15.03 \pm$
1210 1.376 ms) and Saccharin 1x (4hr) (26.21 ± 2.421 ms), Water (19.24 ± 1.620 ms) and Saccharin 1x
1211 (4hr) (26.21 ± 2.421 ms), Saccharin 1x (14.82 ± 1.485 ms) and Saccharin 1x (4hrs) (26.21 ± 2.421
1212 ms), Saccharin 5x (17.30 ± 1.660 ms) and Saccharin 1x (4hr) (26.21 ± 2.421 ms) groups. One-Way
1213 ANOVA, $p < 0.0001$.

1214 For panels 1D, H, I and J: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

1215 All data are shown as mean \pm SEM.

1216 Histological verification of viral delivery at the IC and BLA, as well as locations of whole-cell
1217 patch clamp recording electrode (see Extended Figure 1-1).

1218 Individual IC neurons were classified as burst- and regular-spiking by post-hoc analysis of
1219 responses to rheobase current injections (see Extended Figure 1-2).

1220 The intrinsic properties of burst spiking LIV-VI aIC-BLA projecting neurons are differentially
1221 modulated by taste valence in the context of novelty (see Extended Figures 1-3).

1222

1223 Figure 2: Learned aversive taste memory retrieval decreases the excitability of LIV-VI aIC-
1224 BLA projecting neurons

1225 A) Experimental design of behavioral procedures conducted to compare the intrinsic properties of
1226 LIV-VI aIC-BLA neurons following learned aversive taste memory retrieval (CTA retrieval - n=8
1227 animals, 27 cells), appetitive retrieval for the same tastant (Saccharin 2x - n=5 animals, 20 cells).

1228 B) Mice showed a significantly reduced saccharin consumption following learned aversive
1229 memory retrieval (N=8) compared to appetitive retrieval mice (n=5) group. $p=0.0085$, Mann
1230 Whitney test.

1231 C) Representative traces of LIV-VI aIC-BLA projecting neurons from the two treatment groups.
1232 Scale bars 20 mV vertical and 50ms horizontal from 300 pA step.

1233 D) The excitability of LIV-VI aIC-BLA in the Saccharin 2x group was significantly enhanced
1234 compared to CTA retrieval. Two-way repeated measures ANOVA, Current x Treatment: $p<0.0001$.

1235 E) Representative traces showing action potential measurements for both groups. Scale bar 20 mV
1236 vertical and 2ms horizontal.

1237 F) Representative traces showing the input resistance and sag ratio measurements. Scale bar 10
1238 mV vertical and 100ms horizontal.

1239 G) Action potential amplitude in the CTA retrieval (56.21 ± 0.9978 mV) group was increased
1240 compared to Saccharin 2x (49.14 ± 1.568 mV), $p=0.0005$, Mann Whitney test.

1241 H) Input resistance in the CTA retrieval group (136.4 ± 9.064 M Ω) was significantly decreased
1242 compared to Saccharin 2x (181.1 ± 11.7 M Ω). $p=0.0036$, Unpaired t test.

1243 I) SAG ratio following CTA retrieval (13.41 ± 1.31) was significantly enhanced compared to

1244 Saccharin 2x (7.815 ± 1.176), $p=0.0037$, Unpaired t test.

1245 Data are shown as mean \pm SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

1246 Learned aversive taste memory retrieval decreases the excitability of burst spiking LIV-VI aIC-
1247 BLA neurons (see Extended Figure 2-1).

1248

1249 **Figure 3: Extinction of CTA enhances, whereas reinstatement decreases the excitability of**
1250 **LIV-VI aIC-BLA projecting neurons**

1251 A) Experimental design of behavioral procedures conducted to compare the intrinsic properties of
1252 LIV-VI aIC-BLA neurons following CTA Extinction ($n=5$, animals, 14 cells) and Reinstatement
1253 ($n=3$ animals, 15 cells).

1254 B) The graph showing the reduced aversion following the successful extinction in both treatment
1255 groups.

1256 C) Data showing the saccharin consumption on the test day following successful extinction and
1257 Reinstatement of CTA. CTA reinstated mice showed significantly reduced saccharin consumption
1258 compared to extinguished mice. $p=0.0179$, Mann Whitney test.

1259 D) Representative traces of LIV-VI aIC-BLA projection neurons firing from the two treatment
1260 groups. Scale bars 20 mV and 50ms horizontal from 300 pA step.

1261 E) Excitability in LIV-VI aIC-BLA neurons was significantly different among the treatment
1262 groups. Two-Way repeated measures ANOVA, Current x Treatment: $p<0.0001$.

1263 F) Action potential threshold in the Reinstatement group (-29.43 ± 1.731 mV) was enhanced
1264 compared to Extinction (-36.06 ± 1.481 mV). $p=0.0076$, Unpaired t test.

1265 G) The membrane time constant following Reinstatement (25.48 ± 1.58 ms) was significantly
1266 enhanced compared to Extinction (17.55 ± 2.684 ms, $p=0.047$). $p=0.0153$, Unpaired t test.

1267 For panels 5D-F: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

1268 All data are shown as mean \pm SEM.

1269 Extinction of CTA enhances the excitability of burst spiking LIV-VI aIC-BLA projecting neurons
1270 compared to CTA reinstatement (see Extended Figure 3-1).

1271

1272 Figure 4: Innately aversive taste is correlated with high fAHP, and prolonged conflicting
1273 experiences is correlated with an increased AP half-width in LIV-VI aIC-BLA projecting
1274 neurons

1275 We compared the intrinsic properties of LIV-VI aIC-BLA neurons among the Saccharin 1x (n=5
1276 animals, 20 cells), Quinine 1x (n=4 animals, 19 cells), Saccharin 2x (n=5 animals, 20 cells), CTA
1277 retrieval (n=8, 27 cells), Extinction (n=5 animals, 14 cells) and Reinstatement (n=3 animals, 15
1278 cells) groups.

1279 A) Groups associated with positive taste valence (Saccharin 1x, Saccharin 2x, Extinction),
1280 exhibited significantly increased excitability compared to innate or learned negative taste valence
1281 groups (Quinine 1x, CTA retrieval and Reinstatement). Two-way repeated measures ANOVA,
1282 Current x Treatment: $p<0.0001$; Saccharin 2x vs. CTA retrieval: * p ; Saccharin 2x vs.
1283 Reinstatement: # p ; Saccharin 2x vs Quinine 1x: $p\$$; Saccharin 1x vs. CTA retrieval: p^{\wedge} ; Saccharin
1284 1x vs. Quinine 1x: $p\%$; Saccharin 1x vs reinstatement: $p+$; Extinction vs. CTA retrieval: $p@$;
1285 Extinction vs. Reinstatement: $p\&$; Extinction vs. Quinine 1x: $p-$.

1286 B) fAHP was significantly enhanced in response to Quinine 1x (13.56 ± 1.562 mV) compared to
1287 all other groups. Significant differences were also observed between Saccharin 1x (3.016 ± 0.9423
1288 mV), Saccharin 2x (5.223 ± 0.8217 mV), and CTA retrieval (7.97 ± 1.018 mV, $p=0.0036$).
1289 Extinction (4.731 ± 1.021 mV) and Reinstatement (5.932 ± 1.292 mV). One-Way ANOVA,
1290 $p<0.0001$.

1291 C) Input resistance was significantly different between Saccharin 2x (181.1 ± 11.7 M Ω) and CTA
1292 retrieval (136.4 ± 9.064 M Ω), $p= 0.0352$. Conversely, input resistance in Saccharin 1X ($145.8 \pm$
1293 12.56), Quinine 1X (146 ± 9.094), Extinction (151.1 ± 15.63), and Reinstatement groups was
1294 similar. One-Way ANOVA, $p= 0.0213$.

1295 D) SAG ratio was significantly different between Saccharin 2x (7.815 ± 1.176) and CTA retrieval
1296 (13.41 ± 1.31), $p= 0.0209$. Conversely, SAG ratio in Saccharin 1x (10.89 ± 1.621), Quinine 1x
1297 (12.13 ± 1.23), Extinction (12.37 ± 1.471) and Reinstatement (9.245 ± 0.884) groups was similar
1298 One-Way ANOVA, $p= 0.0286$.

1299 E) Action potential amplitude in the Quinine 1x group (57.11 ± 1.376 mV), and CTA retrieval
1300 (56.21 ± 0.9978 mV), was significantly increased compared to Saccharin 2x (49.14 ± 1.568 mV,
1301 $p= 0.0175$, and 0.0229 , respectively). Conversely, action potential attitude in the Saccharin 1x
1302 (52.03 ± 1.308 mV), Extinction (55.09 ± 2.122 mV) and Reinstatement (53.1 ± 2.906 mV) groups
1303 was similar. One-Way ANOVA, $p = 0.0061$.

1304 F) Action potential half-width following Extinction (0.7386 ± 0.03145 ms) and Reinstatement
1305 (0.8187 ± 0.06929 ms) was elevated compared to Saccharin 1x (0.6005 ± 0.03260 ms), Saccharin
1306 2x (0.5780 ± 0.02994 ms) as well as CTA retrieval (0.5959 ± 0.02080 ms, but no with Quinine 1x
1307 (0.6300 ± 0.03555 ms). One-Way ANOVA, $p = 0.0002$.

1308 G) The membrane time constant in the Saccharin 1x (14.82 ± 1.485 ms) group was significantly
1309 decreased compared to Reinstatement (25.48 ± 1.58 ms, $p= 0.0043$) groups was. Differences
1310 between CTA retrieval (20.96 ± 1.724 ms, $p=0.0189$), Quinine 1x (21.55 ± 1.638 ms), Saccharin
1311 2x (19.28 ± 1.837 ms) and Extinction (17.55 ± 2.684 ms) groups failed to reach significance. One-
1312 Way ANOVA, $p = 0.0047$.

1313

1314 [Figure 5: The intrinsic properties of burst spiking LIV-VI aIC-BLA projecting neurons](#)
1315 [represent taste experience and the probability for further learning](#)

1316 A) Data across all intrinsic properties from BS LIV-VI aIC-BLA neurons of the Saccharin 1x,
1317 Saccharin 2x and Extinction groups were combined and assigned to the Low predictive following
1318 memory group (32 BS cells). Conversely, the intrinsic properties of BS LIV-VI aIC-BLA neurons
1319 from animals having undergone CTA retrieval, 5x Saccharin, and Reinstatement were combined
1320 and assigned to the High predictive following memory group (31 BS cells). The resultant three-
1321 dimensional scatter representation of the two groups encompassed Excitability at 350pA; AP
1322 amplitude, AP halfwidth, AP threshold; fAHP, mAHP, sAHP; IR, Rheobase, RMP, SAG ratio and
1323 τ in BS LIV-VI aIC-BLA neurons. See Extended Figure 5-1.

1324 B) Three-dimensional representation of the contribution of individual parameters (loadings
1325 matrix) to the principal components segregating the two groups of treatments (scores matrix).

1326 PCA variable contributions and component loadings of BS and RS LIV-VI aIC-BLA projecting
1327 neurons in Extended Figure 5-2.

1328 **Extended figures and Tables**

1329

1330 **Extended Figure 1-1: Histological verification of rAAV-mCherry virus expression and**

1331 **locations of whole- cell patch clamp recordings**

1332 **A) A representative image showing the distribution of retrograde injections into the BLA and aIC-**

1333 **BLA projection neuron at aIC.**

1334 **B) Locations showing the retroviral injections sites in the BLA.**

1335 **C) Mean localization of BLA projecting neurons of the agranular aIC used for electrophysiological**

1336 **whole-cell recordings.**

1337

1338 **Extended Figure 1-2: The ratio of burst spiking and regular spiking LIV-VI aIC-BLA**

1339 **projecting neurons changes in relation to the uncertainty associated with taste experiences**

1340 **A) Representative traces from Burst (BS) and Regular (RS) spiking LIV-VI aIC-BLA projecting**

1341 **neurons in response to rheobase current injections. The neurons showing doublets or triplets in**

1342 **response to rheobase current injection were considered BS. The neurons showing single spike in**

1343 **response to rheobase current injection considered RS. Scale bars 20 mV and 100 ms.**

1344 **B) Pie charts showing the change in the ratio of BS vs RS LIV-VI aIC-BLA projection neurons,**

1345 **expressed as a percentage of the sampled population across the Saccharin 1x, Saccharin 2x,**

1346 **Saccharin 5x, CTA retrieval, Extinction, and Reinstatement groups.**

1347 **C) Heat map summary of the change in the ratio of BS vs RS LIV-VI aIC-BLA projection**

1348 **neurons, expressed as a percentage of the sampled population across the six treatment groups.**

1349

1350 Extended Figure 1-3: Appetitive novel taste alters the intrinsic properties of burst spiking

1351 LIV-VI aIC-BLA neurons

1352 We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons among the Cage
1353 control (n=13 cells), Water (n= 11cells), Saccharin 1X (n=17 cells), Quinine 1x (n=9 cells),
1354 Saccharin 5x (n=10 cells) and Saccharin 1x (4hrs, n=6 cells).

1355 A) Excitability in BS LIV-VI aIC-BLA was not significantly different among the treatment groups.
1356 Two-Way repeated measures ANOVA, Current x Treatment: $p < 0.0001$, Group interaction $p =$
1357 0.0666 .

1358 B) fAHP was significantly enhanced in Quinine 1x (13.67 ± 2.681 mV) and Saccharin 5x (11.30
1359 ± 1.727 mV) BS neurons compared to Saccharin 1x BS neurons (2.870 ± 1.044 mV). One-Way
1360 ANOVA, $P = 0.0004$.

1361 C) Action potential amplitude was significantly different between the groups. Cage controls (56.27
1362 ± 1.147 mV), Water (54.21 ± 1.572 mV), Saccharin 1x (51.64 ± 1.473 mV), Quinine 1X ($58.86 \pm$
1363 2.003 mV), Saccharin 5x (58.40 ± 1.812 mV), and Saccharin 1x (4hr) (46.79 ± 4.359 mV). One-
1364 Way ANOVA, $P = 0.0097$.

1365 D) Action potential half-width in BS LIV-VI aIC-BLA neurons of the Saccharin 1x (4 hrs.) group
1366 (0.8850 ± 0.05943 ms) was increased compared to the Saccharin 1x (1hr) group - $0.5976 \pm$
1367 0.03555 ms. One-Way ANOVA, $P = 0.0139$.

1368 E) Action potential threshold was not significantly different between the groups. Cage control (-
1369 31.83 ± 2.971 mV), Water (-29.27 ± 2.060 mV), Saccharin 1x (-30.73 ± 2.385 mV), Quinine 1x (-
1370 29.35 ± 3.071 mV), Saccharin 5x (-30.38 ± 2.493 mV), and Saccharin 1x (4hr) (-34.61 ± 2.174

1371 mV). One-Way ANOVA, $P= 0.7652$.

1372 F) Input resistance was similar among the different treatment groups. Cage control (118.4 ± 9.771
1373 $M\Omega$), Water ($136.5 \pm 14.40 M\Omega$), Saccharin 1x ($146.6 \pm 14.22 M\Omega$), Quinine 1x (139.2 ± 16.86
1374 $M\Omega$), Saccharin 5x ($156.1 \pm 22.85 M\Omega$), and Saccharin 1x (4hr) ($154.9 \pm 22.41 M\Omega$). One-Way
1375 ANOVA, $P= 0.6304$.

1376 G) SAG ratio was not significantly different between the groups. Cage control (14.91 ± 2.195),
1377 Water (8.751 ± 2.021), Saccharin 1x (11.67 ± 1.790), Quinine 1x (14.15 ± 2.159), Saccharin 5x
1378 (11.92 ± 3.395), and Saccharin 1x (4hr) (14.99 ± 2.770). One-Way ANOVA, $P= 0.2232$.

1379 H) Membrane time constant was significantly different among the treatment groups. Cage control
1380 (14.71 ± 1.944 ms), Water (18.03 ± 2.309 ms), Saccharin 1x (14.27 ± 1.666 ms), Quinine 1x (23.21
1381 ± 2.717 ms), Saccharin 5x (17.11 ± 2.296 ms), and Saccharin 1x (4hr) (26.09 ± 5.331 ms). One-
1382 Way ANOVA, $P= 0.0321$.

1383 Data are shown as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

1384

1385 [Extended Figure 2-1: Learned aversive taste memory retrieval decreases the excitability](#)
1386 [of burst spiking LIV-VI aIC-BLA neurons](#)

1387 We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons following Saccharin
1388 2xs (BS=13, RS=7, cells) and CTA memory retrieval (BS=12, RS=15, cells).

1389 A) Excitability in BS LIV-VI aIC-BLA neurons was significantly reduced in the CTA retrieval
1390 group compared to Saccharin 2x. Two-Way repeated measures ANOVA, Current x Treatment:
1391 $p < 0.0001$.

1392 B) Input resistance in BS LIV-VI aIC-BLA neurons was significantly enhanced in the Saccharin
1393 2x ($180.3 \pm 15.15 \text{ M}\Omega$) compared to CTA retrieval ($110.9 \pm 12.98 \text{ M}\Omega$). Unpaired t test, $p=0.0022$.

1394 C) Action potential amplitude in BS LIV-VI aIC-BLA neurons was significantly increased in the
1395 CTA retrieval group compared to Saccharin 2x ($46.18 \pm 1.666 \text{ mV}$), and CTA retrieval ($57.87 \pm$
1396 1.678 mV). Mann Whitney test, $p<0.0001$.

1397 D) SAG ratio in BS LIV-VI aIC-BLA neurons was significantly decreased in the Saccharin 2x
1398 (7.017 ± 1.317) compared to CTA retrieval (16.8 ± 1.869). Mann Whitney test, $p=0.0005$.

1399 E) Representative traces of RS LIV-VI aIC-BLA neurons firing from the two treatments. Scale
1400 bars 20 mV vertical and 50ms horizontal in response to 150 pA step current.

1401 F) Excitability in RS LIV-VI aIC-BLA neurons was similar in the CTA retrieval and Saccharin 2x.
1402 Two-Way repeated measures ANOVA, Current x Treatment: $p=0.0953$.

1403 G) Input resistance in RS LIV-VI aIC-BLA neurons was not significantly different in between the
1404 groups. Saccharin 2x ($182.6 \pm 19.62 \text{ M}\Omega$), and CTA retrieval ($156.7 \pm 10.11 \text{ M}\Omega$). Mann Whitney
1405 test, $p>0.9999$.

1406 H) SAG ratio in RS LIV-VI aIC-BLA neurons was not significantly different between the groups.
1407 Saccharin 2x (9.297 ± 2.347), and CTA retrieval (10.71 ± 1.536). Mann Whitney test, $p=0.5815$.

1408 I) Action potential amplitude in RS LIV-VI aIC-BLA neurons was not significantly different
1409 between the groups. Saccharin 2x ($54.62 \pm 2.058 \text{ mV}$), and CTA retrieval ($54.89 \pm 1.13 \text{ mV}$). Mann
1410 Whitney test, $p>0.9999$.

1411 J) AP half-width in RS LIV-VI aIC-BLA neurons was significantly reduced following CTA
1412 memory retrieval ($0.5633 \pm 0.01703 \text{ ms}$) compared to the Saccharin 2x ($0.6614 \pm 0.04149 \text{ ms}$).

1413 Mann Whitney test, $p= 0.0200$.

1414 K) Membrane time constant was similar in both treatment groups. Saccharin 2x RS ($18.36 \pm$
1415 2.842ms), and CTA memory retrieval RS ($24.08 \pm 2.023 \text{ ms}$). Mann Whitney test, $p= 0.0777$.

1416 Data are shown as mean \pm SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

1417

1418 [Extended Figure 3-1: Extinction of CTA enhances, excitability of burst spiking LIV-VI](#)
1419 [aIC-BLA projecting neurons](#)

1420 We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons following the
1421 Extinction (BS=11, RS=3, cells) and Reinstatement (BS=10, RS=5, cells).

1422 A) Excitability in BS LIV-VI aIC-BLA was significantly enhanced in Extinction group comparing
1423 to Reinstatement. Two-Way repeated measures ANOVA, Current x Treatment: $p<0.0001$.

1424 B) sAHP in BS LIV-VI aIC-BLA neurons was significantly enhanced in the Extinction group ($-$
1425 $2.104 \pm 0.4466 \text{ mV}$) compared to Reinstatement ($-3.804 \pm 1.339 \text{ mV}$) neurons. Mann Whitney
1426 test, $p=0.0230$.

1427 C) Action potential threshold in BS LIV-VI aIC-BLA neurons was significantly reduced in the
1428 Extinction group ($-37.41 \pm 1.636 \text{ mV}$) compared to Reinstatement ($-27.5 \pm 2.195 \text{ mV}$). Unpaired
1429 t test, $p= 0.0016$.

1430 D) Input resistance in BS LIV-VI aIC-BLA neurons was similar in the two treatment groups.
1431 Extinction ($131.1 \pm 13.93 \text{ M}\Omega$) and Reinstatement BS ($157.4 \pm 10.56 \text{ M}\Omega$). Mann Whitney test,
1432 $p= 0.1321$.

1433 E) SAG ratio in BS LIV-VI aIC-BLA neurons was enhanced following Extinction (13.69 ± 1.541)

1434 neurons compared to Reinstatement BS (9.124 ± 1.03). Unpaired t test, $p= 0.0262$.

1435 F) Membrane time constant in BS LIV-VI aIC-BLA neurons was significantly reduced in the
1436 Extinction group (14.52 ± 2.714 ms) compared to Reinstatement (26.93 ± 1.893) neurons. Mann
1437 Whitney test, $p= 0.0062$.

1438 G) Representative traces of RS LIV-VI aIC-BLA firing from two treatment groups. Scale bars 20
1439 mV vertical and 50 ms horizontal in response to 150 pA current step.

1440 H) Excitability of RS LIV-VI aIC-BLA neurons in both treatment groups.

1441 I) Input resistance in RS LIV-VI aIC-BLA neurons was similar in the Extinction (224.2 ± 21.29
1442 $M\Omega$) and Reinstatement ($221.2 \pm 18.9 M\Omega$) groups.

1443 J) SAG ratio in RS LIV-VI aIC-BLA neurons was not different between the Extinction ($7.515 \pm$
1444 2.666) and Reinstatement (9.486 ± 1.846) groups.

1445 K) Membrane time constant in RS LIV-VI aIC-BLA neurons was not different between the
1446 Extinction (28.69 ± 2.138 ms) and Reinstatement groups (22.58 ± 2.632 ms).

1447

1448 [Extended Figure 5-1: PCA showing Burst vs Regular spiking LIV-VI aIC-BLA neurons all](#)
1449 [range of excitability vs 350 pA only](#)

1450 A) PCA of BS and RS LIV-VI aIC-BLA neurons all range of excitability (50-350 pA and all other
1451 intrinsic properties measured). Sampled population across six treatment groups (Saccharin 1x,
1452 Saccharin 2x, Saccharin 5x, CTA retrieval, Extinction, Reinstatement).

1453 B) PCA of BS and RS LIV-VI aIC-BLA neurons excitability of 350 pA only and all other intrinsic
1454 properties measured. Sampled population across six treatment groups (Saccharin 1x, Saccharin 2x,
1455 Saccharin 5x, CTA retrieval, Extinction, Reinstatement).

1456

1457 **Extended Figure 5-2: PCA variable contributions and component loadings of burst- and**
1458 **regular-spiking LIV-VI aIC-BLA projecting neurons**

1459 A) Column chart demonstrating the individual and cumulative proportion of the variance
1460 accounted by principal components following PCA of BS LIV-VI aIC-BLA projecting neurons in
1461 the two groups of treatments (Saccharin 1x, Saccharin 2x, Extinction vs. CTA retrieval, 5x
1462 Saccharin, Reinstatement).

1463 B) Table summarizing the contribution of individual variables (loadings) to the coordinate value
1464 of the principal components segregating the two groups (score).

1465 C) Communalities table, demonstrating the amount of variance in each variable that is accounted
1466 for by the extraction of principal components. Initial communalities are estimates of the variance
1467 in each variable accounted for by all components or factors (=1.00).

1468

1469 **Table 1: Summary of LIV-VI aIC-BLA intrinsic properties**

1470 Values are expressed in mean \pm SEM. The number of cells is in parentheses. Statistical analysis
1471 was performed by One-way ANOVA Post-hoc Tukey's and Dunn's multiple comparisons.
1472 Student's t-test was performed for the comparison between two groups. RMP - resting membrane
1473 potential, fAHP, mAHP and sAHP - fast, medium, slow after hyperpolarization potentials,
1474 respectively. AP Thresh - action potential threshold, AP Amp - action potential amplitude. AP half-

1475 width - action potential half-width. Data are shown as mean \pm SEM. * p <0.05, ** p <0.01,
1476 *** p <0.001, **** p <0.0001, with respect to the corresponding symbols.

1477

1478 **Table 2: Summary of LI-III aIC-BLA intrinsic properties**

1479 Values are expressed as mean \pm SEM. The number of cells is in parentheses. Statistical analysis
1480 was performed by Student's t-test. RMP - resting membrane potential, mAHP - medium after
1481 hyperpolarization potentials. AP Thresh - action potential threshold, AP Amp - action potential
1482 amplitude. AP half-width - action potential half-width.

1483

1484 **Table 3: Summary of BS LIV-VI aIC-BLA intrinsic properties**

1485 Values are expressed in mean \pm SEM. The number of cells is in parentheses. Statistical analysis
1486 was performed by One-way ANOVA Post-hoc Tukey's and Dunn's multiple comparisons.
1487 Student's t-test was performed for the comparison between two groups. RMP - resting membrane
1488 potential, fAHP, mAHP and sAHP - fast, medium, slow after hyperpolarization potentials,
1489 respectively. AP Thresh - action potential threshold, AP Amp - action potential amplitude. AP half-
1490 width - action potential half-width.

1491

1492 **Table 4: Summary of RS LIV-VI aIC-BLA intrinsic properties**

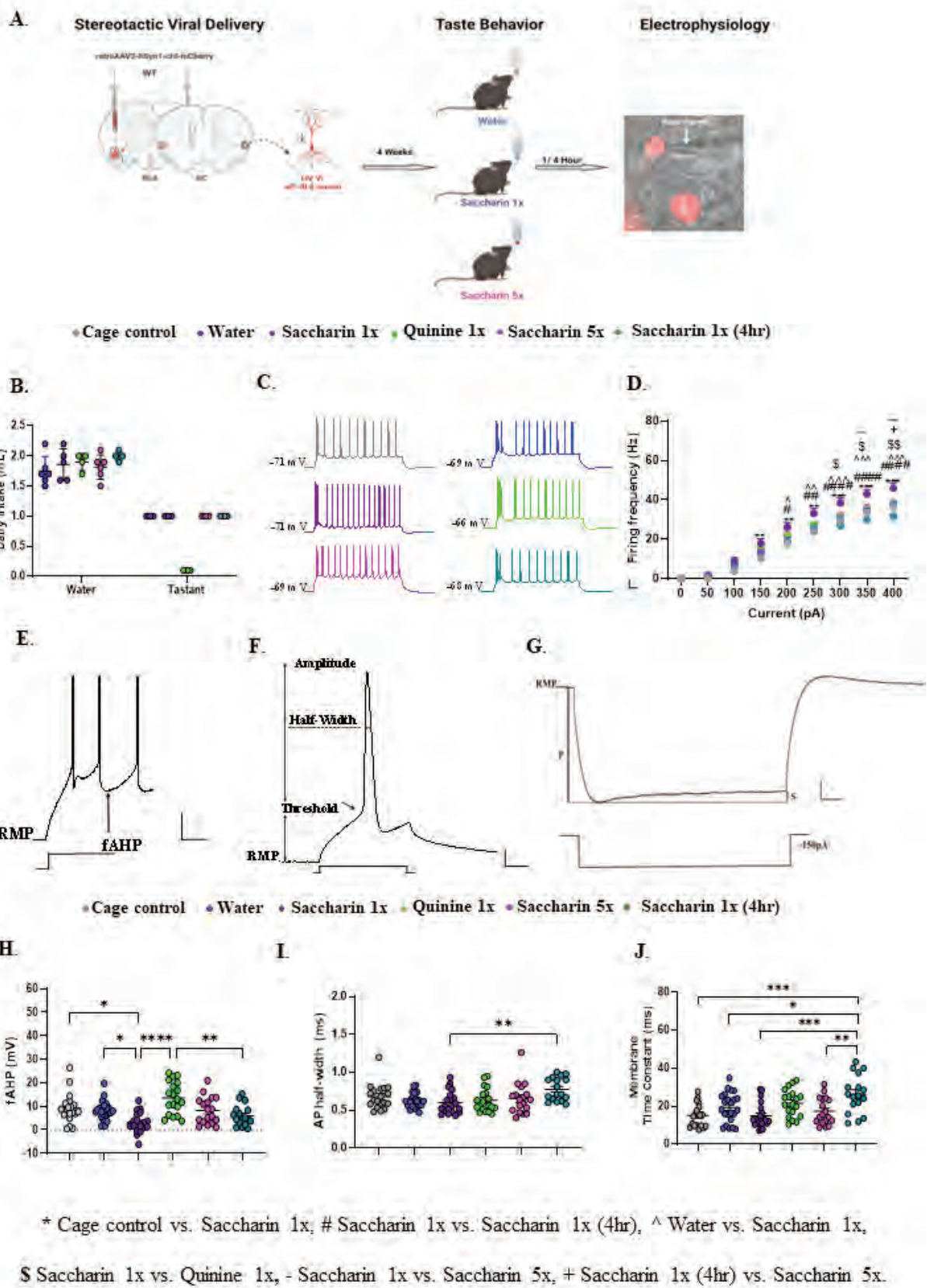
1493 Values are expressed in mean \pm SEM. The number of cells is in parentheses. Statistical analysis
1494 was performed by One-way ANOVA Post-hoc Tukey's and Dunn's multiple comparisons.
1495 Student's t-test was performed for the comparison of two groups. RMP - resting membrane
1496 potential, fAHP, mAHP and sAHP - fast, medium, slow after hyperpolarization potentials,

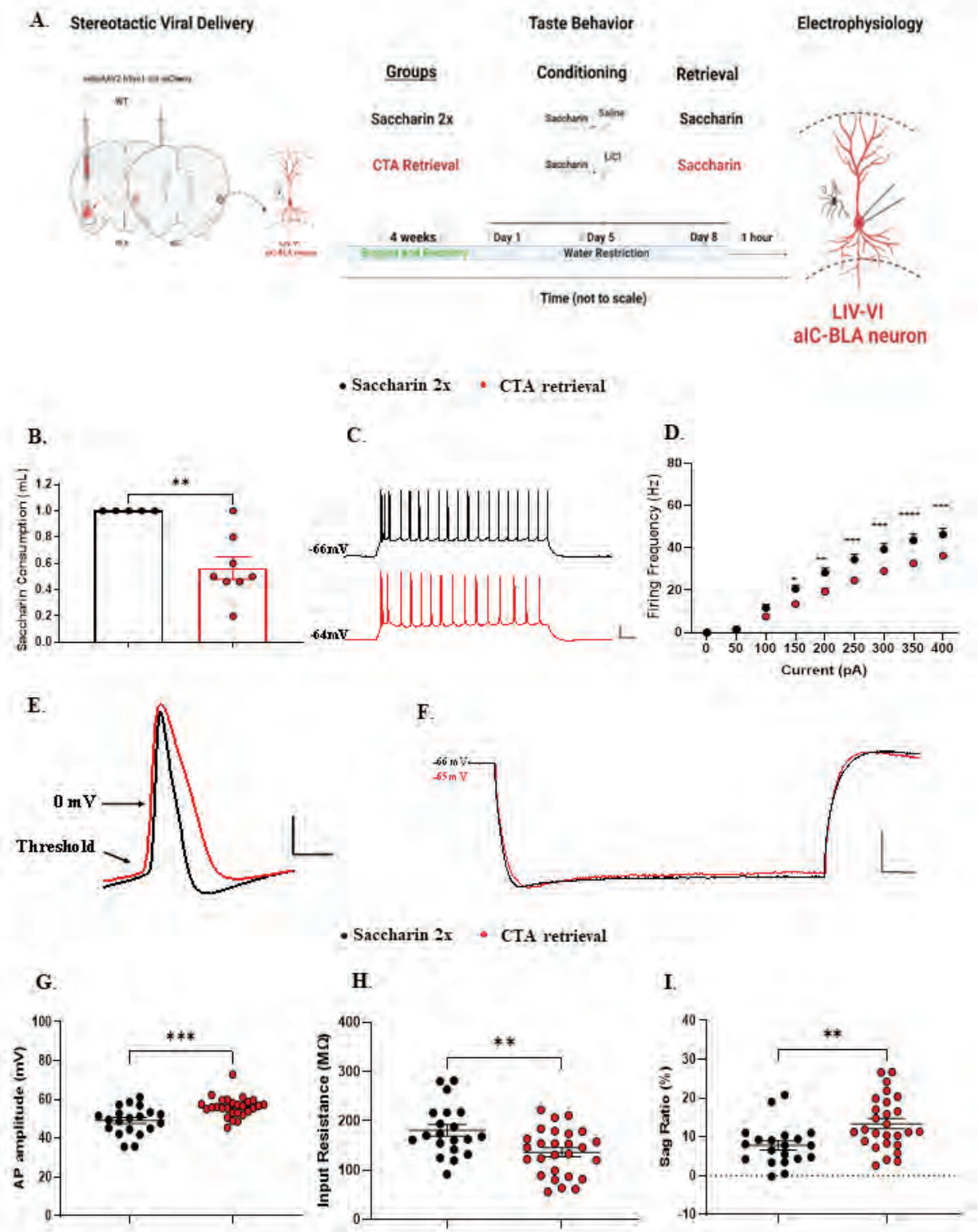
1497 respectively. AP Thresh - action potential threshold, AP Amp - action potential amplitude. AP half-

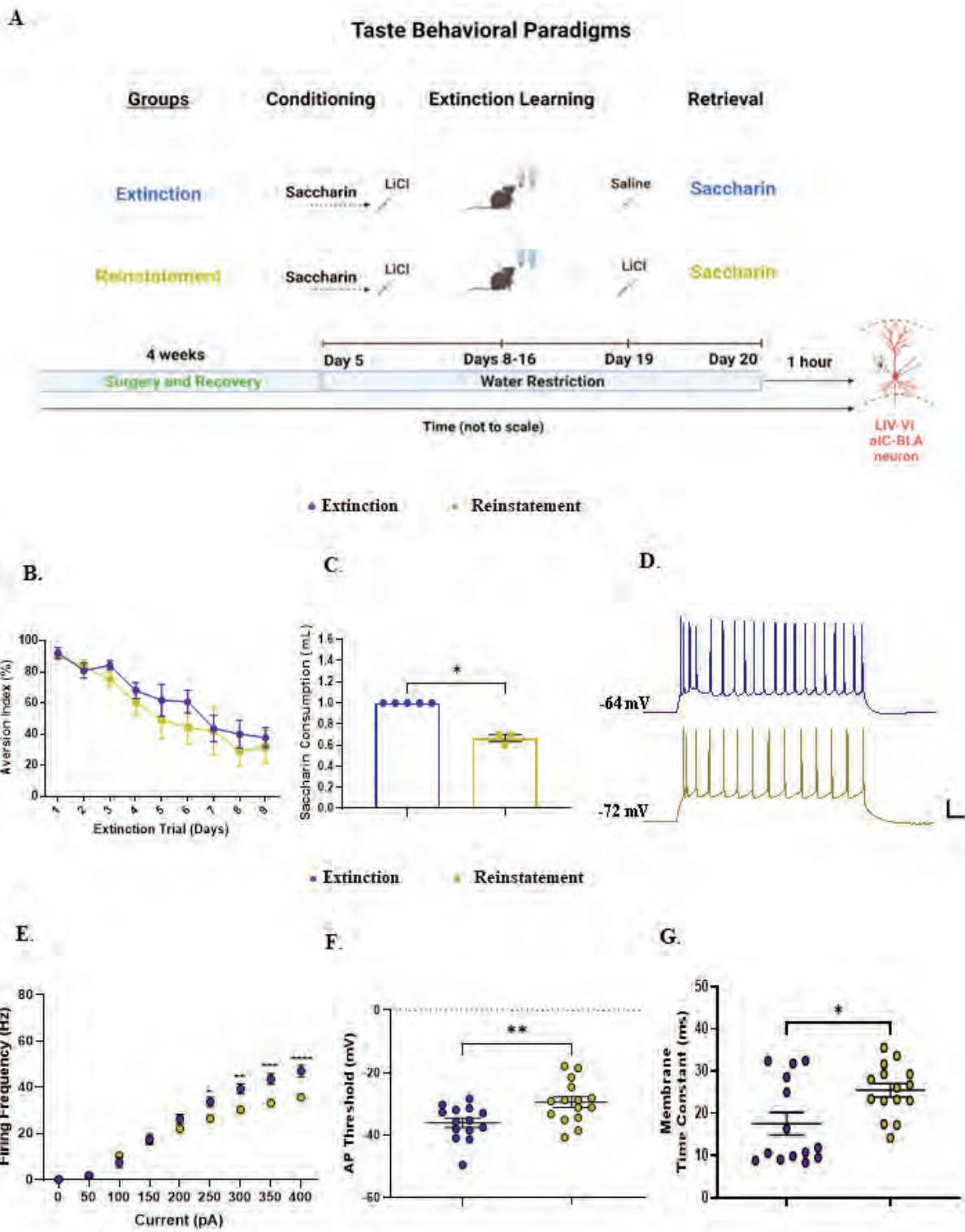
1498 width - action potential half-width.

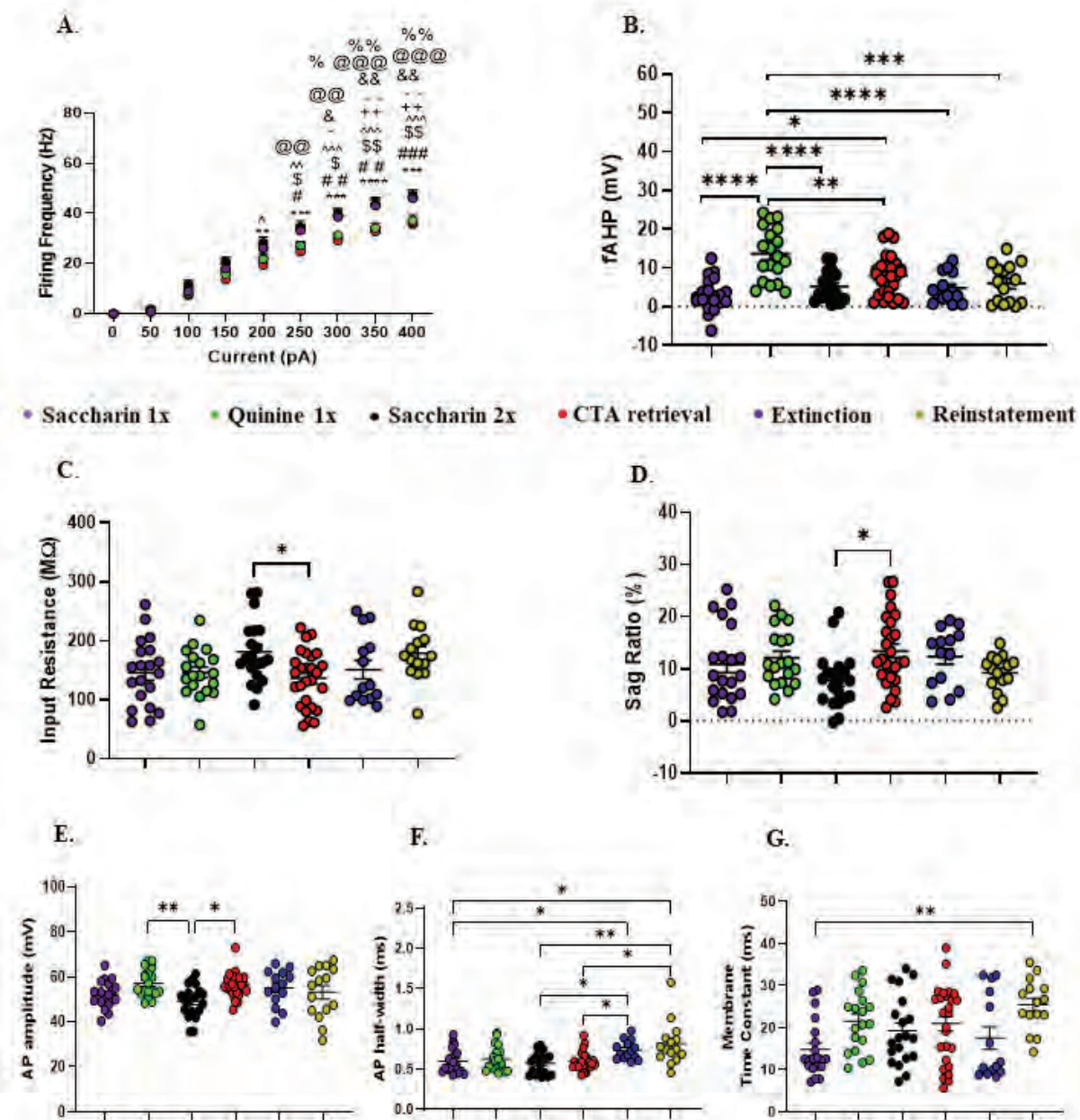
1499

1500 [Table 5: Statistics Table.](#)





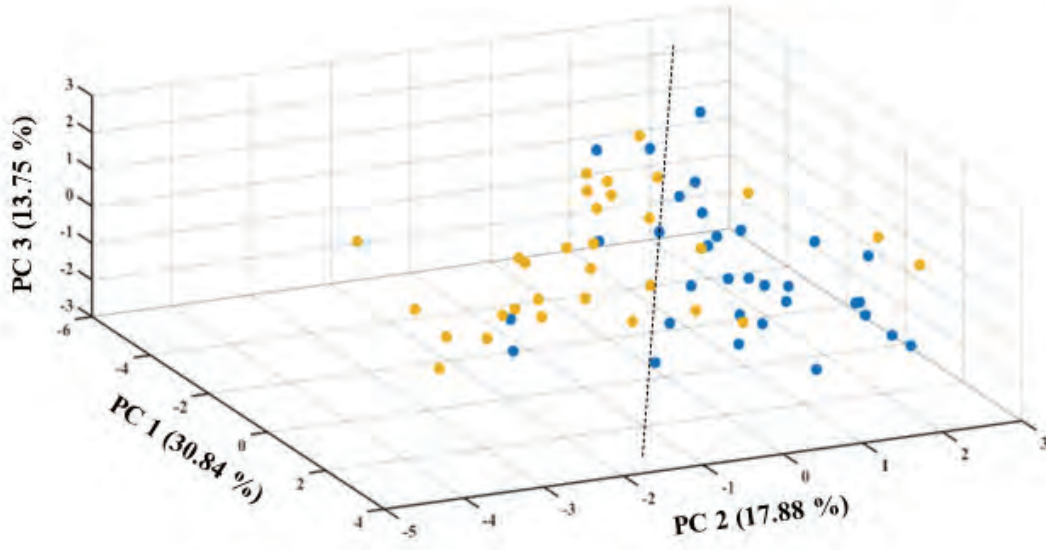




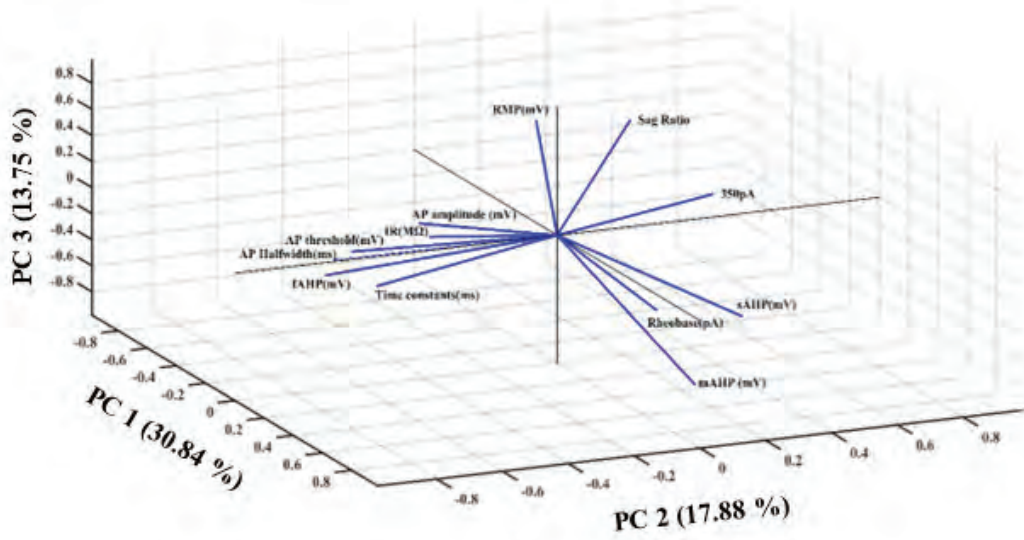
* Saccharin 2x vs. CTA retrieval, # Saccharin 2x vs. Reinstatement, \$ Saccharin 2x vs Quinine, ^ Saccharin 1x vs. CTA retrieval, % Saccharin 1x vs. Quinine 1x, + Saccharin 1x vs reinstatement, @ Extinction vs. CTA retrieval, & Extinction vs. Reinstatement, - Extinction vs. Quinine 1x.

A.

- Low Predictive Following memory
- High Predictive Following memory



B.



Groups	RMP (mV)	fAHP (mV)	mAHP (mV)	sAHP (mV)	Input Resistance (MΩ)	Sag ratio (%)	Time constant (ms)	AP threshold (mV)	AP Amplitude (mV)	AP half- width (ms)	Rheobase (pA)
LIV-VI aIC-BLA Cage control	-68.28 ± 0.8506 (19)	9.191 ± 1.449 (19) ** ##	-3.73 ± 0.4241 (19)	-1.881 ± 0.3376 (19)	120.7 ± 7.686 (19)	12.41 ± 1.938 (19)	15.03 ± 1.376 (19)	-30.76 ± 2.139 (19)	56.28 ± 0.8818 (19) ^^	0.6774 ± 0.03816 (19)	87.47 ± 9.127 (19)
LIV-VI aIC-BLA Water	-69.3 ± 0.9051 (23)	8.15 ± 0.8288 (23) **	-5.535 ± 0.6754 (23)	-3.277 ± 0.4603 (23)	139.1 ± 9.021(23)	8.909 ± 1.306 (23)	19.24 ± 1.62 (23)	-31.05 ± 1.30 (23)	52.43 ± 1.034 (23)	0.6243 ± 0.02021 (23)	85 ± 11.76 (23)
LIV-VI aIC-BLA Saccharin 1x	-69.21 ± 0.94 (20)	3.016 ± 0.9423 (20) #####	-4.17 ± 0.4542 (20)	-2.521 ± 0.2735 (20)	145.8 ± 12.56 (20)	10.89 ± 1.621 (20)	14.82 ± 1.485 (20) #	-30.9 ± 2.141 (20)	52.03 ± 1.308 (20)	0.6005 ± 0.0326 (20)	74.25 ± 11.39 (20)
LIV-VI aIC-BLA Quinine 1x	-67.32 ± 1.092 (19)	13.56 ± 1.562 (19) SSSS ~	-5.858 ± 0.5613 (18)	-3.634 ± 0.3632 (18)	146 ± 9.094 (19)	12.13 ± 1.23 (19)	21.55 ± 1.638 (19)	-29.74 ± 1.989 (19)	57.11 ± 1.376 (19) *****	0.6 ± 0.03555 (19)	69.89 ± 8.932 (19)
LIV-VI aIC-BLA Saccharin 5x	-66.53 ± 1.358 (18)	8.158 ± 1.356 (18) ** ##	-3.999 ± 0.653 (18)	-2.695 ± 0.5083 (18)	144.6 ± 14.68 (18)	9.392 ± 2.127 (18)	17.3 ± 1.66 (18)	-32.66 ± 1.783 (18)	56.48 ± 1.337 (18)	0.6539 ± 0.04814 (18)	78.61 ± 10.75 (18)
LIV-VI aIC-BLA Saccharin 1x (4hr)	-69.47 ± 0.7569 (17)	5.989 ± 1.074 (17) **	-4.411 ± 0.8962 (17)	-2.631 ± 0.6949 (17)	153.9 ± 11.10 (17)	10.97 ± 1.475 (17)	26.21 ± 2.421 (17) *** SSS % &&	-31.03 ± 1.511 (17)	52.24 ± 2.311 (17)	0.7765 ± 0.03641 (17) **	78.88 ± 9.274 (17)
LIV-VI aIC-BLA Saccharin 2x	-70.79 ± 1.242 (20)	5.223 ± 0.8217 (20) ###	-5.301 ± 0.7863 (20)	-3.351 ± 0.3798 (20)	181.1 ± 11.7 (20)	7.815 ± 1.176 (20)	19.28 ± 1.837 (20)	-32.85 ± 1.447 (20)	49.14 ± 1.568 (20) ###	0.578 ± 0.02994 (20)	69.6 ± 10.71 (20)
LIV-VI aIC-BLA CTA Retrieval	-68.78 ± 0.8419 (27)	7.97 ± 1.018 (27) ##	-5.213 ± 0.4544 (27)	-2.69 ± 0.3064 (27)	136.4 ± 9.064 (27) ^^	13.41 ± 1.131 (27) ^^	20.96 ± 1.724 (27) *	-31.61 ± 2.68 (27)	56.21 ± 0.9978 (27) ^^^	0.5959 ± 0.0208 (27)	90.44 ± 17.56 (27)
LIV-VI aIC-BLA Extinction	-65.98 ± 1.457 (14)	4.731 ± 1.021 (14)	-5.076 ± 0.6981 (14)	-2.895 ± 0.6547 (14)	151.1 ± 15.63 (14)	12.37 ± 1.471 (14)	17.55 ± 2.684 (14) ~	-36.06 ± 1.481 (14) ~	55.09 ± 2.122 (14) ^	0.7386 ± 0.03145 (14)	69.21 ± 7.454 (14)
LIV-VI aIC-BLA Reinstatement	-68.57 ± 0.936 (15)	5.932 ± 1.292 (15)	-5.673 ± 0.4288 (15)	-3.612 ± 0.3033 (15)	178.7 ± 12.1 (15)	9.245 ± 0.884 (15)	25.48 ± 1.58 (15) **	-29.43 ± 1.731 (15)	53.1 ± 2.906 (15)	0.8187 ± 0.06929 (15)	67.6 ± 8.753 (15)

\$Vs. Cage Control * Vs. Saccharin 1x ^ Vs. Saccharin 2x ~ Vs. Reinstatement

%Vs. Water # Vs. Quinine 1x & Vs. Saccharin 5x

Groups	RMP (mV)	mAHP (mV)	Input resistance (M Ω)	Sag ratio (%)	Time constant (ms)	AP thresh (mV)	AP Amp (mV)	AP half-width (ms)	Rheobase (pA)
LI-III aIC-BLA Water	-70.84 \pm 1.09 (14)	-2.411 \pm 0.6767 (14)	136.4 \pm 16.85 (14)	3.172 \pm 1.082 (14)	11.17 \pm 1.169 (14)	-31.35 \pm 1.547 (14)	56 \pm 1.719 (14)	0.6557 \pm 0.03222 (14)	140.7 \pm 31.83 (14)
LI-III aIC-BLA Saccharin 1x	-72.27 \pm 1.212 (15)	-1.752 \pm 0.4953 (15)	131.6 \pm 13.14 (15)	3.997 \pm 0.9166 (15)	11.56 \pm 1.672 (15)	-31.44 \pm 1.621 (15)	56.33 \pm 1.323 (15)	0.684 \pm 0.02767 (15)	136.1 \pm 26.44(15)
LI-III aIC-BLA Saccharin 2x	-73.44 \pm 1.295 (15)	-2.165 \pm 0.684 (15)	142.5 \pm 13.17 (15)	5.793 \pm 1.449 (15)	18.63 \pm 1.9 (15)	-31.47 \pm 2.511 (15)	49.76 \pm 2.065 (15)	0.564 \pm 0.03708 (15)	104.7 \pm 25.03 (15)
LI-III aIC-BLA CTA Retrieval	-72 \pm 1.117 (17)	-3.087 \pm 2.914 (17)	171.1 \pm 22.28 (17)	6.307 \pm 1.368 (17)	18.94 \pm 1.667 (17)	-34.24 \pm 1.445 (17)	48.58 \pm 1.472 (17)	0.5518 \pm 0.02698 (17)	114.3 \pm 31.57 (17)

Groups	RMP (mV)	fAHP (mV)	mAHP (mV)	sAHP (mV)	Input resistance (M Ω)	Sag ratio (%)	Time constant (ms)	AP thresh (mV)	AP Amp (mV)	AP half-width (ms)	Rheobase (pA)
BS LIV-VI aIC-BLA Cage control	-68.28 ± 0.9705 (13)	9.192 ± 2.061 (13)	-4.194 ± 0.5072 (13)	-2.075 ± 0.4500 (13)	118.4 ± 9.771 (13)	14.91 ± 2.195 (13)	14.71 ± 1.944 (13)	-31.83 ± 2.971 (13)	56.27 ± 1.147 (13)	0.6692 ± 0.05460 (13)	74.54 ± 8.471 (13)
BS LIV-VI aIC-BLA Water	69.00 ± 1.639 (11)	7.800 ± 1.607 (11)	-4.870 ± 0.8838 (11)	-2.826 ± 0.6069 (11)	136.5 ± 14.40 (11)	8.751 ± 2.021 (11)	18.03 ± 2.309 (11)	-29.27 ± 2.060 (11)	54.21 ± 1.572 (11)	0.6736 ± 0.03111 (11)	85.91 ± 13.44 (11)
BS LIV-VI aIC-BLA Saccharin 1x	-68.80 ± 1.065 (17)	2.870 ± 1.044 (17)	-4.339 ± 0.5083 (17)	-2.564 ± 0.3032 (17)	146.6 ± 14.22 (17)	11.67 ± 1.790 (17)	14.27 ± 1.666 (17)	-30.73 ± 2.385 (17)	51.64 ± 1.473 (17)	0.5976 ± 0.03555 (17)	63.65 ± 7.679 (17)
BS LIV-VI aIC-BLA Quinine 1x	-67.37 ± 1.682 (9)	13.67 ± 2.681 (9) ##	-6.131 ± 0.6514 (8)	-3.58 ± 0.5788 (8)	139.2 ± 16.86 (9)	14.15 ± 2.159 (9)	23.21 ± 2.717 (9)	-29.35 ± 3.071 (9)	58.86 ± 2.003 (9)	0.6378 ± 0.0491 (9)	59.56 ± 12.28 (9)
BS LIV-VI aIC-BLA Saccharin 5x	-67.20 ± 1.624 (10)	11.30 ± 1.727 (10) ##	-5.174 ± 0.8427 (10)	-3.609 ± 0.7205 (10)	156.1 ± 22.85 (10)	11.92 ± 3.395 (10)	17.11 ± 2.296 (10)	-30.38 ± 2.493 (10)	58.40 ± 1.812 (10)	0.7140 ± 0.07349 (10)	76.60 ± 15.32 (10)
BS LIV-VI aIC-BLA Saccharin 1x (4hr)	-68.20 ± 1.293 (6)	3.433 ± 0.9245 (6)	-6.693 ± 1.442 (6)	-3.130 ± 1.637 (6)	154.9 ± 22.41 (6)	14.99 ± 2.770 (6)	26.09 ± 5.331 (6)	-34.61 ± 2.174 (6)	46.79 ± 4.359 (6)	0.8850 ± 0.05943 (6) #	60.83 ± 11.36 (6)
BS LIV-VI aIC-BLA Saccharin 2x	-71.33 ± 1.641 (13)	4.169 ± 0.9225 (13)	-5.368 ± 0.9616 (13)	-3.226 ± 0.4899 (13)	180.3 ± 15.15 (13) **	7.017 ± 1.317 (13) ***	19.77 ± 2.447 (13)	-34.20 ± 1.987 (13)	46.18 ± 1.666 (13) ***	0.5331 ± 0.03522 (13)	77.54 ± 15.69 (13)
BS LIV-VI aIC-BLA CTA Retrieval	-67.37 ± 1.21 (12)	5.473 ± 1.464 (12)	-4.633 ± 0.5831 (12)	-1.932 ± 0.4462 (12)	110.9 ± 12.98 (12)	16.8 ± 1.869 (12)	17.06 ± 2.608 (12)	-34.28 ± 1.771 (12)	57.87 ± 1.678 (12)	0.6367 ± 0.03961 (12)	88.75 ± 9.847 (12)
BS LIV-VI aIC-BLA Extinction	-67.36 ± 1.43 (11)	3.943 ± 1.111 (11)	-4.816 ± 0.8447 (11)	-2.104 ± 0.4466 (11) ~	131.1 ± 13.93 (11)	13.69 ± 1.541 (11)	14.52 ± 2.714 (11) ~ ~	-37.41 ± 1.636 (11) ~ ~	57.3 ± 2.023 (11)	0.7155 ± 0.03674 (11)	81 ± 6.932 (11)
BS LIV-VI aIC-BLA Reinstatement	-68.88 ± 1.163 (10)	6.432 ± 1.737 (10)	-5.243 ± 0.5853 (10)	-3.804 ± 1.339 (10)	157.4 ± 10.56 (10)	9.124 ± 1.03 (10)	26.93 ± 1.893 (10)	-27.5 ± 2.195 (10)	57.36 ± 3.001 (10)	0.769 ± 0.03494 (10)	73.9 ± 12.45 (10)

* BS LV/VI aIC-BLA Saccharin 2x vs. CTA Retrieval, * p<0.05, ** p<0.01, *** p<0.001.

~ BS LV/VI aIC-BLA Extinction vs. Reinstatement, ~ p<0.05, ~ ~ p<0.01, ~ ~ ~ p<0.001.

BS LV/VI aIC-BLA Saccharin 1x vs. Quinine 1x, Saccharin 5x and Saccharin 1x (4hr),

p<0.05, ## p<0.01.

Groups	RMP (mV)	fAHP (mV)	mAHP (mV)	sAHP (mV)	Input resistance (M Ω)	Sag ratio (%)	Time constant (ms)	AP thresh (mV)	AP Amp (mV)	AP half- width (ms)	Rheobase (pA)
RS LIV-VI aIC-BLA Cage control	-68.29 ± 1.830 (6)	9.188 \pm 1.351 (6)	-2.725 \pm 0.6462 (6)	-1.460 \pm 0.4409 (6)	125.7 \pm 13.03 (6)	6.993 \pm 3.030 (6)	15.73 \pm 1.333 (6)	-28.44 \pm 2.166 (6)	56.30 \pm 1.422 (6)	0.6950 \pm 0.03170 (6)	115.5 \pm 18.62 (6)
RS LIV-VI aIC-BLA Water	-69.57 \pm 0.9422 (12)	8.472 \pm 0.6792 (12)	-6.144 \pm 1.013 (12)	-3.690 \pm 0.6876 (12)	141.5 \pm 11.75 (12)	9.735 \pm 1.400 (12)	20.35 \pm 2.321 (12)	-32.67 \pm 1.561 (12)	50.79 \pm 1.238 (12)	0.5792 \pm 0.01928 (12)	84.17 \pm 19.48 (12)
RS LIV-VI aIC-BLA Saccharin 1x	-71.57 ± 1.120 (3)	3.840 \pm 2.530 (3)	-3.210 \pm 0.9005 (3)	-2.277 \pm 0.7297 (3)	141.3 \pm 28.45 (3)	6.455 \pm 3.099 (3)	17.95 \pm 2.856 (3)	-31.86 \pm 5.650 (3)	54.23 \pm 2.660 (3)	0.6167 \pm 0.09939 (3)	134.3 \pm 58.52 (3)
RS LIV-VI aIC-BLA Quinine 1x	-67.28 ± 1.505 (10)	11.63 \pm 1.616 (10)	-5.639 \pm 0.8918 (10)	-3.678 \pm 0.4895 (10)	151.4 \pm 9.055 (10)	10.31 \pm 1.115 (10)	23.6 \pm 1.77(10)	-30.1 \pm 2.732 (10)	55.53 \pm 1.845 (10)	0.6230 \pm 0.0535 (10)	79.20 \pm 12.73 (10)
RS LIV-VI aIC-BLA Saccharin 5x	-65.70 ± 2.378 (8)	4.235 \pm 1.141 (8)	-2.530 \pm 0.7960 (8)	-1.553 ± 0.4915 (8)	130.1 \pm 16.85 (8)	6.237 \pm 1.910 (8)	17.54 \pm 2.561 (8)	-35.50 \pm 2.302 (8)	54.09 \pm 1.734 (8)	0.5788 \pm 0.05034 (8)	81.13 ± 15.88 (8)
RS LIV-VI aIC-BLA Saccharin 1x (4hr)	-70.17 \pm 0.9075 (11)	7.383 \pm 1.439 (11)	-3.165 \pm 0.9897 (11)	-2.359 \pm 0.6647 (11)	153.3 \pm 12.96 (11)	8.782 \pm 1.389 (11)	26.28 \pm 2.596 (11)	-28.43 \pm 1.652 (11)	51.66 \pm 3.053 (11)	0.7773 \pm 0.04702 (11)	88.73 \pm 12.25 (11)
RS LIV-VI aIC-BLA Saccharin 2x	-69.79 ± 1.921 (7)	7.180 \pm 1.402 (7)	-5.016 \pm 1.460 (7)	-3.581 \pm 0.6324 (7)	182.6 \pm 19.62 (7)	9.297 \pm 2.347 (7)	18.36 \pm 2.842 (7)	-30.36 \pm 1.638 (7)	54.62 \pm 2.058 (7)	0.6614 \pm 0.04149 (7)	54.86 \pm 8.207 (7)
RS LIV-VI aIC-BLA CTA Retrieval	-69.9 \pm 1.116 (15)	9.967 \pm 1.216 (15)	-5.667 \pm 0.6647 (15)	-3.297 \pm 0.3599(15)	156.7 \pm 10.11 (15)	10.71 \pm 1.536 (15)	24.08 \pm 2.023 (15)	-33.9 \pm 1.132 (15)	54.89 \pm 1.13 (15)	0.5633 \pm 0.01703 (15) *	91.8 \pm 31.15 (15)

* RS LV/VI aIC-BLA Saccharin 1x vs. CTA Retrieval, *p<0.05.

Groups (Table 4 Cont.)	RMP (mV)	fAHP (mV)	mAHP (mV)	sAHP (mV)	Input resistance (MΩ)	Sag ratio (%)	Time constant (ms)	AP thresh (mV)	AP Amp (mV)	AP half- width (ms)	Rheobase (pA)
RS LIV-VI aIC-BLA Extinction	-60.93 ± 3.263 (3)	7.620 ± 1.907 (3)	-6.027 ± 1.062 (3)	-5.797 ± 1.997 (3)	224.2 ± 21.29 (3)	7.515 ± 2.666 (3)	28.69 ± 2.138 (3)	-31.1 ± 1.372 (3)	46.98 ± 4.432 (3)	0.8233 ± 0.02603 (3)	36.67 ± 13.33 (3)
RS LIV-VI aIC-BLA Reinstatement	-67.95 ± 1.725 (5)	4.932 ± 1.893 (5)	-6.532 ± 0.3344 (5)	-3.228 ± 0.3214 (5)	221.2 ± 18.9 (5)	9.486 ± 1.846 (5)	22.58 ± 2.632 (5)	-33.31 ± 2.035 (5)	44.58 ± 4.569 (5)	0.918 ± 0.203 (5)	55 ± 6.885 (5)

* RS LV/VI aIC-BLA Saccharin 1x vs. CTA Retrieval, *p<0.05.

STATISTICS TABLE		
Figure	Statistical Test	Results
FIGURE 1		
Figure 1 D	Two-way repeated measures ANOVA Post-hoc Tukey's multiple comparisons LIV-VI aIC-BLA neurons F-I curve Cage control Water Saccharin 1x Quinine 1x Saccharin 5x Saccharin 1x (4hr)	<p><u>ANOVA Results:</u> Treatment; $p=0.0057$, $F(5, 110)=3.491$ Current; $p<0.0001$, $F(8, 880)=1276$ Interaction; $p<0.0001$, $F(40, 880)=4.141$</p> <p><u>Multiple Comparisons:</u></p> <p>0pA Cage control vs. Water Mean difference=0.000 Cage control vs. Saccharin 1x Mean difference=0.000 Cage control vs. Quinine 1x Mean difference=0.000 Cage Control vs. Saccharin 5x Mean difference=0.000 Cage Control vs. Saccharin 1x (4hr) Mean difference=0.000 Water vs. Saccharin 1x Mean difference=0.000 Water vs. Quinine 1x Mean difference=0.000 Water vs. Saccharin 5x Mean difference=0.000 Water vs. Saccharin 1x (4hr) Mean difference=0.000 Saccharin 1x vs. Quinine 1x Mean difference=0.000 Saccharin 1x vs. Saccharin 5x Mean difference=0.000 Saccharin 1x vs. Saccharin 1x (4hr) Mean difference=0.000 Quinine 1x vs. Saccharin 5x Mean difference=0.000 Quinine 1x vs. Saccharin 1x (4hr) Mean difference=0.000 Saccharin 5x vs. Saccharin 1x (4hr) Mean difference=0.000</p> <p>50pA Cage control vs. Water $p=0.9902$, $q=0.8645$, $df=990.0$; Cage control vs. Saccharin 1x $p=0.9993$, $q=0.5005$, $df=990.0$; Cage Control vs. Quinine 1x $p>0.9999$, $q=0.2597$, $df=990.00$; Cage control vs. Saccharin 5x $p=0.9451$, $q=1.281$, $df=990.00$; Cage control vs. Saccharin 1x(4hr) $p=0.9922$, $q=0.8240$, $df=990$; Water vs. Saccharin 1x $p=0.9999$, $q=0.3522$, $df=990$ Water vs. Quinine 1x</p>

	<p>p=0.9946, q=0.5927, df=990; Water vs. Saccharin 5x p=0.9984, q=0.4868, df=990; Water vs. Saccharin 1x (4hr) p>0.9999, q=0.02212, df=990; Saccharin 1x vs. Quinine 1x p>0.9999, q=0.2374, df=990; Saccharin 1x vs. Saccharin 5x p=0.9931, q=0.8029, df=990; Saccharin 1x vs. Saccharin 1x (4hr) p=0.9999, q=0.3478, df=990; Quinine 1x vs. Saccharin 5x p=0.9790, q=1.024, df=990; Quinine 1x vs. Saccharin 1x (4hr) p=0.9986, q=0.5716, df=990; Saccharin 5x vs. Saccharin 1x (4hr) p=0.9996, q=0.4320, df=990;</p> <p>100pA Cage control vs. Water p= 0.8652, q= 1.610, df= 990.0; Cage control vs. Saccharin 1x p= 0.2233, q= 3.159, df= 990.0; Cage Control vs. Quinine 1x p =0.4580, q= 2.563, df=990.00; Cage control vs. Saccharin 5x p= 0.6454, q= 2.163, df=990.00; Cage control vs. Saccharin 1x(4hr) p= 0.5930, q= 2.275, df=990; Water vs. Saccharin 1x p=0.8437, q=1.677, df=990 Water vs. Quinine 1x p=0.9742, q=1.073, df=990; Water vs. Saccharin 5x p=0.9969, q=0.6743, df=990; Water vs. Saccharin 1x (4hr) p=0.9926, q=0.8138, df=990; Saccharin 1x vs. Quinine 1x p=0.9987, q=0.5625, df=990; Saccharin 1x vs. Saccharin 5x p=0.9867, q=0.9250, df=990; Saccharin 1x vs. Saccharin 1x (4hr) p=0.9945, q=0.7652, df=990; Quinine 1x vs. Saccharin 5x p=0.9998, q=0.3658, df=990; Quinine 1x vs. Saccharin 1x (4hr) p>0.9999, q=0.2164, df=990; Saccharin 5x vs. Saccharin 1x (4hr) p>0.9999, q=0.1422, df=990;</p> <p>150pA Cage control vs. Water p= 0.8024, q= 1.793, df= 990.0; Cage control vs. Saccharin 1x p= 0.0085, q= 4.836, df= 990.0; Cage Control vs. Quinine 1x p =0.1482, q= 3.431, df=990.00; Cage control vs. Saccharin 5x p= 0.2881, q= 2.970, df=990.00; Cage control vs. Saccharin 1x(4hr)</p>
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	<p>p= 0.5463, q= 2.374, df=990; Water vs. Saccharin 1x p=0.1962, q=3.248, df=990 Water vs. Quinine 1x p=0.8009, q=1.797, df=990; Water vs. Saccharin 5x p=0.9345, q=1.337, df=990; Water vs. Saccharin 1x (4hr) p=0.9953, q=0.7397, df=990; Saccharin 1x vs. Quinine 1x p=0.9297, q=1.361, df=990; Saccharin 1x vs. Saccharin 5x p=0.8142, q=1.762, df=990; Saccharin 1x vs. Saccharin 1x (4hr) p=0.5843, q=2.293, df=990; Quinine 1x vs. Saccharin 5x p=0.9997, q=0.4145, df=990; Quinine 1x vs. Saccharin 1x (4hr) p=0.9843, q=0. 0.9603, df=990; Saccharin 5x vs. Saccharin 1x (4hr) P=0.9989, q=0.5448, df=990;</p> <p>200pA Cage Control vs. Water p=0.9968, q=0.6822, df=990.0; Cage Control vs. Saccharin 1x p=0.0038, q=0.6822, df=990.0; Cage Control vs. Quinine 1x p=0.5360, q=2.396, df=990.0; Cage Control vs. Saccharin 5x p=0.5556, q=2.354, df=990.0; Cage Control vs. Saccharin 1x (4hr) p=0.9876, q=0.9108, df=990.0; Water vs. Saccharin 1x p=0.0116, q=4.708, df=990.0; Water vs. Quinine 1x p=0.7904, q=4.708, df=990.0; Water vs. Saccharin 5x p=0.8042, q=1.789, df=990.0; Water vs. Saccharin 1x (4hr) p>0.9999, q=0.2894, df=990.0; Saccharin 1x vs. Quinine 1x p=0.3853, q=2.727, df=990.0; Saccharin 1x vs. Saccharin 5x p=0.3979, q=2.698, df=990.0; Saccharin 1x vs. Saccharin 1x (4hr) p=0.0457, q=4.083, df=990.0; Quinine 1x vs. Saccharin 5x p>0.9999, q=0.008880, df=990.0; Quinine 1x vs. Saccharin 1x (4hr) p=0.9172, q=1.417, df=990.0; Saccharin 5x vs. Saccharin 1x (4hr) p=0.9233, q=1.391, df=990.0;</p> <p>250pA Cage control vs. Water p=0.9988, q=,0.5590 df=990.0; Cage control vs. Saccharin 1x p=0.0011, q=5.603, df=990.0; Cage Control vs. Quinine 1x</p>
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	<p>p=0.7555, q=1.912, df=990.0; Cage Control vs. Saccharin 5x p=0.7478, q=1.931, df=990.0; Cage Control vs. Saccharin 1x (4hr) p>0.9999, q=0.1164, df=990.0; Water vs. Saccharin 1x p=0.0026, q=5.304, df=990.0; Water vs. Quinine 1x p=0.9113, q=1.442, df=990.0; Water vs. Saccharin 5x p=0.9051, q=1.468, df=990.0; Water vs. Saccharin 1x (4hr) p=0.9972, q=0.6633, df=990.0; Saccharin 1x vs. Quinine 1x p=0.1000, q=3.666, df=990.0; Saccharin 1x vs. Saccharin 5x p=0.1180, q=3.570, df=990.0; Saccharin 1x vs. Saccharin 1x (4hr) p=0.0013, q=5.559, df=990.0; Quinine 1x vs. Saccharin 5x p>0.9999, q=0.04456, df=990.0; Quinine 1x vs. Saccharin 1x (4hr) p=0.7292, q=1.975, df=990.0; Saccharin 5x vs. Saccharin 1x (4hr) p=0.7215, q=1.993, df=990.0;</p> <p>300pA Cage Control vs. Water p=0.9993, q=0.4987, df=990.0; Cage Control vs. Saccharin 1x p=0.0005, q=5.903, df=990.0; Cage Control vs. Quinine 1x p=0.9022, q=1.479, df=990.0; Cage Control vs. Saccharin 5x p=0.7419, q=1.945, df=990.0; Cage Control vs. Saccharin 1x (4hr) p=0.9641, q=1.158, df=990.0; Water vs. Saccharin 1x p=0.0009, q=5.679, df=990.0; Water vs. Quinine 1x p=0.9766, q=1.049, df=990.0; Water vs. Saccharin 5x p=0.8854, q=1.542, df=990.0; Water vs. Saccharin 1x (4hr) p=0.8386, q=1.692, df=990.0; Saccharin 1x vs. Quinine 1x p=0.0232, q=4.405, df=990.0; Saccharin 1x vs. Saccharin 5x p=0.0716, q=3.851, df=990.0; Saccharin 1x vs. Saccharin 1x (4hr) p<0.0001, q=6.905, df=990.0; Quinine 1x vs. Saccharin 5x p=.9994, q=0.4860, df=990.0; Quinine 1x vs. Saccharin 1x (4hr) p=0.4433, q=2.596, df=990.0; Saccharin 5x vs. Saccharin 1x (4hr) p=0.2645, q=3.035, df=990.0;</p> <p>350pA Cage Control vs. Water</p>
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	<p>p=0.9973, q=0.6556, df=990.0; Cage Control vs. Saccharin 1x p=0.0003, q=6.069, df=990.0; Cage Control vs. Quinine 1x p=0.9972, q=0.6596, df=990.0; Cage Control vs. Saccharin 5x p=0.8294, q=1.719, df=990.0; Cage Control vs. Saccharin 1x (4hr) p=0.7347, q=1.962, df=990.0; Water vs. Saccharin 1x p=0.0009, q=5.694, df=990.0; Water vs. Quinine 1x p>0.9999, q=0.03478, df=990.0; Water vs. Saccharin 5x p=0.9650, q=1.151, df=990.0; Water vs. Saccharin 1x (4hr) p=0.4043, q=2.683, df=990.0; Saccharin 1x vs. Quinine 1x p=0.0020, q=5.401, df=990.0; Saccharin 1x vs. Saccharin 5x p=0.0328, q=4.244, df=990.0; Saccharin 1x vs. Saccharin 1x (4hr) p<0.0001, q=7.879, df=990.0; Quinine 1x vs. Saccharin 5x p=0.9747, q=1.068, df=990.0; Quinine 1x vs. Saccharin 1x (4hr) p=0.4400, q=2.603, df=990.0; Saccharin 5x vs. Saccharin 1x (4hr) p=0.1105, q=3.609, df=990.0;</p> <p>400pA Cage Control vs. Water p=0.9988, q=0.5513, df=990.0; Cage Control vs. Saccharin 1x p=0.0004, q=5.939, df=990.0; Cage Control vs. Quinine 1x p=0.9987, q=0.5623, df=990.0; Cage Control vs. Saccharin 5x p=0.9132, q=1.435, df=990.0; Cage Control vs. Saccharin 1x (4hr) p=0.3765, q=2.748, df=990.0; Water vs. Saccharin 1x p=0.0009, q=5.663, df=990.0; Water vs. Quinine 1x p>0.9999, q=0.03710, df=990.0; Water vs. Saccharin 5x p=0.9845, q=0.9564, df=990.0; Water vs. Saccharin 1x (4hr) p=0.1551, q=3.402, df=990.0; Saccharin 1x vs. Quinine 1x p=0.0021, q=5.369, df=990.0; Saccharin 1x vs. Saccharin 5x p=0.0233, q=4.403, df=990.0; Saccharin 1x vs. Saccharin 1x (4hr) p<0.0001, q=8.548, df=990.0; Quinine 1x vs. Saccharin 5x p=0.9894, q=0.8800, df=990.0; Quinine 1x vs. Saccharin 1x (4hr) p=0.1832, q=3.294, df=990.0; Saccharin 5x vs. Saccharin 1x (4hr)</p>
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		p=0.0435, q=4.108, df=990.0.
Figure 1 B	<p>One -Way Anova</p> <p>Water consumption the before the test</p> <p>Water Saccharin 1x Quinine 1x Saccharin 5x Saccharin 1x (4hrs)</p>	<p><u>ANOVA results:</u></p> <p>F=0.9766 P= 0.4424 R squared, 0.1634</p>
Figure 1H	<p>One-way ANOVA</p> <p>Kruskal-Wallis test Post-hoc Dunn's multiple comparisons test</p> <p>LIV-VI aIC-BLA neurons fAHP</p> <p>Cage control Water Saccharin 1x Quinine 1x Saccharin 5x Saccharin 1x (4hr)</p>	<p><u>ANOVA results:</u> Kruskal-Wallis test, p<0.0001; Kruskal-Wallis statistic,29.91. <u>Multiple Comparisons:</u></p> <p>Cage Control vs. Water p >0.9999, z= 0.2306; Cage Control vs. Saccharin 1x p=0.0136, z= 3.318; Cage Control vs. Quinine 1x p >0.9999, z= 1.809; Cage Control vs. Saccharin 5x p >0.9999, z= 0.4824; Cage Control vs. Saccharin 1x (4hr) p>0.9999, z= 1.648; Water vs. Saccharin 1x p= 0.0177, z= 3.243; Water vs. Quinine 1x p= 0.5054, z=2.124; Water vs. Saccharin 5x p>0.9999, z=0.2771; Water vs. Saccharin 1x (4hr) p>0.9999, z=1.497; Saccharin 1x vs. Quinine 1x p<0.0001, z=5.150; Saccharin 1x vs. Saccharin 5x p=0.0807, z=2.783; Saccharin 1x vs. Saccharin 1x (4hr) p>0.9999, z=1.554; Quinine 1x vs. Saccharin 5x p=0.3511 z=2.267; Quinine 1x vs. Saccharin 1x (4hr) p=0.0099, z=3.406; Saccharin 5x vs. Saccharin 1x (4hr) p>0.9999, z= 1.1583</p>
Figure II	<p>One-way ANOVA</p> <p>Kruskal-Wallis test Post-hoc Dunn's multiple comparisons test</p> <p>LIV-VI aIC-BLA neurons Action Potential Half-width</p>	<p><u>ANOVA results:</u> Kruskal-Wallis test, p=0.0125; Kruskal-Wallis statistic,14.54. <u>Multiple Comparisons:</u></p> <p>Cage Control vs. Water p >0.9999, z= 0.7692; Cage Control vs. Saccharin 1x p>0.9999, z= 1.627;</p>

	<p>Cage control Water Saccharin 1x Quinine 1x Saccharin 5x Saccharin 1x (4hr)</p>	<p>Cage Control vs. Quinine 1x p >0.9999, z=0.9868 ; Cage Control vs. Saccharin 5x p >0.9999, z= 0.6540; Cage Control vs. Saccharin 1x (4hr) P=0.8292, z= 1.917; Water vs. Saccharin 1x p >0.9999, z= 0.9244; Water vs. Quinine 1x p>0.9999, z=0.2636; Water vs. Saccharin 5x p>0.9999, z=0.07420; Water vs. Saccharin 1x (4hr) p=0.0905, z=2.746; Saccharin 1x vs. Quinine 1x p>0.9999, z=0.6271 Saccharin 1x vs. Saccharin 5x p=>0.9999, z=0.9418; Saccharin 1x vs. Saccharin 1x (4hr) p=0.0065, z=3.519; Quinine 1x vs. Saccharin 5x p>0.9999z=0.3194; Quinine 1x vs. Saccharin 1x (4hr) p=0.0605, z=2.876; Saccharin 5x vs. Saccharin 1x (4hr) p= 0.1721, z= 2.528</p>
<p>Figure 1J</p>	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons LIV-VI aIC-BLA neurons Membrane Time Constant Cage control Water Saccharin 1x Quinine 1x Saccharin 5x Saccharin 1x (4hr)</p>	<p><u>ANOVA results:</u> Treatment; p<0.0001. F (5, 110) = 6.094; <u>R squared, 0.2169;</u> <u>Multiple Comparisons:</u> Cage Control vs. Water p = 0.4608, q= 2.566, df=110; Cage Control vs. Saccharin 1x p >0.9999, q= 0.1233, df=110; Cage Control vs. Quinine 1x p = 0.0864, q= 3.798, df=110; Cage Control vs. Saccharin 5x p = 0.9398, q= 1.306, df=110; Cage Control vs. Saccharin 1x (4hr) p = 0.0003, q= 6.326, df=110; Water vs. Saccharin 1x p = 0.3890, q= 2.731, df=110; Water vs. Quinine 1x p = 0.9184, q= 1.408, df=110; Water vs. Saccharin 5x p = 0.9628, q= 1.163, df=110; Water vs. Saccharin 1x (4hr) p = 0.0488, q= 4.115, df=110; Saccharin 1x vs. Quinine 1x p = 0.0639, q= 3.969, df=110; Saccharin 1x vs. Saccharin 5x p = 0.9101, q= 1.443, df=110; Saccharin 1x vs. Saccharin 1x (4hr) p = 0.0002, q= 6.521, df=110; Quinine 1x vs. Saccharin 5x p = 0.5180, q= 2.440, df=110; Quinine 1x vs. Saccharin 1x (4hr) p = 0.4302, q= 2.635, df=110;</p>

		Saccharin 5x vs. Saccharin 1x (4hr) p = 0.0081, q= 4.975, df=110;
FIGURE 2		
Figure 2B	Two-tailed Unpaired t-test Saccharin consumption on the test day Saccharin 2x CTA retrieval	t-test Results: <u>Mann-Whitney test, p= 0.0085,</u> <u>Mann-Whitney U, 2.500</u>
Figure 2D	Two-way repeated measures ANOVA Post-hoc Šídák's multiple comparisons test LIV-VI aIC-BLA neurons F-I curve Saccharin 2x CTA retrieval	<u>ANOVA Results:</u> Treatment; p<0.0014, F (1, 45) =11.60 Current; p<0.0001, F (8, 360) =483.3 Interaction; p<0.0001, F (8, 360) =9.398 <u>Multiple Comparisons:</u> 0pA Saccharin 2x vs. CTA retrieval Mean difference=0.000 50pA Saccharin 2x vs. CTA retrieval p>0.9999, t=0.1045, df=405.0; 100pA Saccharin 2x vs. CTA retrieval p=0.5860, t=1.682, df=405.0; 150pA Saccharin 2x vs. CTA retrieval p=0.0286, t=2.964, df=405.0; 200pA Saccharin 2x vs. CTA retrieval p=0.0019, t=3.738, df=405.0; 250pA Saccharin 2x vs. CTA retrieval p=0.0005, t=4.090, df=405.0; 300pA Saccharin 2x vs. CTA retrieval p=0.0002, t=4.280, df=405.0; 350pA Saccharin 2x vs. CTA retrieval p<0.0001, t=4.517, df=405.0; 400pA Saccharin 2x vs. CTA retrieval p=0.0003, t=4.161, df=405.0
Figure 2G	Two-tailed Unpaired t-test LIV-VI aIC-BLA neurons Action Potential Amplitude Saccharin 2x CTA retrieval	p=0.0002 t=3.983 df=45 Difference between means=7.080±1.777 R squared, 0.2607
Figure 2H	Two-tailed Unpaired t-test LIV-VI aIC-BLA neurons Input Resistance Saccharin 2x CTA retrieval	p=0.0036 t=3.072 df=45 Difference between means= 44.75±14.57 R squared=0.1734
Figure 2I	Two-tailed Unpaired t-test	p=0.0037

	LIV-VI aIC-BLA neurons SAG Ratio Saccharin 2x CTA retrieval	t=3.060 df=45 Difference between means=5.597±1.829 R squared=0.1723
FIGURE 3		
Figure 3B	Two-way ANOVA Post-hoc Šídák's multiple comparisons test	ANOVA Results: Treatment, P=0.068, F (1, 54) = 3.466; Interaction, P=0.9697, F (8, 54) = 0.2803.
Figure 3C	Two-tailed Unpaired t-test Saccharin consumption on the test day Extinction Reinstatement	<u>t-test Results:</u> Mann-Whitney test, p= 0.0179; Mann-Whitney U, 0
Figure 3E	Two-way repeated measures ANOVA Post-hoc Šídák's multiple comparisons test LIV-VI aIC-BLA neurons F-I curve	<u>ANOVA Results:</u> Treatment; p=0.0013, F (3, 72) =5.837 Current; p<0.0001, F (1.959, 141.0) =802.5 Interaction; p<0.0001, F (24, 567) =6.468 <u>Multiple Comparisons:</u> 0pA Extinction vs. Reinstatement Mean difference=0.000; 50pA Extinction vs. Reinstatement p >0.9999, t= 0.1718, df= 243.0; 100pA Extinction vs. Reinstatement p= 0.8899, t= 1.237, df= 243.0; 150pA Extinction vs. Reinstatement p >0.9999, t= 0.2492, df= 243.0; 200pA Extinction vs. Reinstatement p= 0.5553, t= 1.723, df= 243.0; 250pA Extinction vs. Reinstatement p= 0.0341, t= 2.919, df= 243.0; 300pA Extinction vs. Reinstatement p= 0.0030, t= 3.636, df= 243.0; 350pA Extinction vs. Reinstatement p= 0.0003, q= 4.203, df= 243.0; 400pA Extinction vs. Reinstatement p <0.0001, t= 4.578, df=243.0.

<p>Figure 3F</p>	<p>Two-tailed Unpaired t-test</p> <p>LIV-VI aIC-BLA neurons Action Potential Threshold</p> <p>Extinction Reinstatement</p>	<p><u>t-test Results:</u> p= 0.0076 t=2.887 df=27 Difference between means; 6.621 ± 2.293 R squared, 0.2359</p>
<p>Figure 3G</p>	<p>Two-tailed Unpaired t-test</p> <p>LIV-VI aIC-BLA neurons Membrane Time Constant</p> <p>Extinction Reinstatement</p>	<p><u>t-test Results:</u> p= 0.0153 t=2.589 df=27 Difference between means ;7.931 ± 3.064 R squared; 0.1988</p>
<p>FIGURE 4</p>		
<p>Figure 4A</p>	<p>Two-way repeated measures ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons F-I Curve</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p><u>ANOVA results:</u> Treatment; p=0.0014, F (5, 109) =4.281 Current; p<0.0001, F (1.990, 216.9) =1218 Interaction; p<0.0001, F (40, 872) =4.978</p> <p><u>Multiple Comparisons:</u> 0pA Saccharin 1x vs. Quinine 1x Mean difference=0.000 Saccharin 1x vs. Saccharin 2x Mean difference=0.000 Saccharin 1x vs. CTA Retrieval Mean difference=0.000 Saccharin 1x vs. Extinction Mean difference=0.000 Saccharin 1x vs. Reinstatement Mean difference=0.000 Quinine 1x vs. Saccharin 2x Mean difference=0.000 Quinine 1x vs. CTA Retrieval Mean difference=0.000 Quinine 1x vs. Extinction Mean difference=0.000 Quinine 1x vs. Reinstatement Mean difference=0.000 Saccharin 2x vs. CTA Retrieval Mean difference=0.000 Saccharin 2x vs. Extinction Mean difference=0.000 Saccharin 2x vs. Reinstatement Mean difference=0.000 CTA Retrieval vs. Extinction Mean difference=0.000 CTA Retrieval vs. Reinstatement Mean difference=0.000 Extinction vs. Reinstatement Mean difference=0.000</p>

	<p>50pA Saccharin 1x vs. Quinine 1x $p > 0.9999$, $q = 0.2195$, $df = 981.0$; Saccharin 1x vs. Saccharin 2x $p = 0.9993$, $q = 0.5023$, $df = 981.0$; Saccharin 1x vs. CTA Retrieval $p = 0.9998$, $q = 0.3831$, $df = 981.0$; Saccharin 1x vs. Extinction $p = 0.9986$, $q = 0.5691$, $df = 981.0$; Saccharin 1x vs. Reinstatement $p = 0.9999$, $q = 0.3539$, $df = 981.0$; Quinine 1x vs. Saccharin 2x $p = 0.9960$, $q = 0.7153$, $df = 981.0$; Quinine 1x vs. CTA Retrieval $p = 0.9981$, $q = 0.6123$, $df = 981.0$; Quinine 1x vs. Extinction $p = 0.9945$, $q = 0.7626$, $df = 981.0$; Quinine 1x vs. Reinstatement $p = 0.9988$, $q = 0.5535$, $df = 981.0$; Saccharin 2x vs. CTA Retrieval $p > 0.9999$, $q = 0.1553$, $df = 981.0$; Saccharin 2x vs. Extinction $p > 0.9999$, $q = 0.1132$, $df = 981.0$; Saccharin 2x vs. Reinstatement $p > 0.9999$, $q = 0.1112$, $df = 981.0$; CTA Retrieval vs. Extinction $p > 0.9999$, $q = 0.2589$, $df = 981.0$; CTA Retrieval vs. Reinstatement $p > 0.9999$, $q = 0.02435$, $df = 981.0$; Extinction vs. Reinstatement $p > 0.9999$, $q = 0.2084$, $df = 981.0$;</p> <p>100pA Saccharin 1x vs. Quinine 1x $p = 0.9991$, $q = 0.5199$, $df = 981.0$; Saccharin 1x vs. Saccharin 2x $p = 0.8659$, $q = 1.608$, $df = 981.0$; Saccharin 1x vs. CTA Retrieval $p = 0.9941$, $q = 0.7759$, $df = 981.0$; Saccharin 1x vs. Extinction $p = 0.9933$, $q = 0.7975$, $df = 981.0$; Saccharin 1x vs. Reinstatement $p = 0.9924$, $q = 0.8188$, $df = 981.0$; Quinine 1x vs. Saccharin 2x $p = 0.6709$, $q = 2.107$, $df = 981.0$; Quinine 1x vs. CTA Retrieval $p > 0.9999$, $q = 0.2082$, $df = 981.0$; Quinine 1x vs. Extinction $p > 0.9999$, $q = 0.3161$, $df = 981.0$; Quinine 1x vs. Reinstatement $p = 0.9431$, $q = 1.292$, $df = 981.0$; Saccharin 2x vs. CTA Retrieval $p = 0.4875$, $q = 2.499$, $df = 981.0$; Saccharin 2x vs. Extinction $p = 0.6016$, $q = 2.257$, $df = 981.0$; Saccharin 2x vs. Reinstatement $p = 0.9970$, $q = 0.6698$, $df = 981.0$; CTA Retrieval vs. Extinction $p > 0.9999$, $q = 0.1487$, $df = 981.0$; CTA Retrieval vs. Reinstatement</p>
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		<p>p= 0.8745, q=1.579, df=981.0; Extinction vs. Reinstatement p= 0.8967, q=1.500, df=981.0</p> <p>150pA Saccharin 1x vs. Quinine 1x p= 0.9491, q=1.258, df=981.0; Saccharin 1x vs. Saccharin 2x p= 0.8741, q=1.258, df=981.0; Saccharin 1x vs. CTA Retrieval p= 0.3926, q=2.71, df=981.0; Saccharin 1x vs. Extinction p>0.9999, q=0.2459, df=981.0; Saccharin 1x vs. Reinstatement p= 0.9985, q=0.5798, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.5798, q=2.818, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.937, q=1.324, df=981.0; Quinine 1x vs. Extinction p= 0.9882, q=0.9008, df=981.0; Quinine 1x vs. Reinstatement p= 0.9983, q=0.5934, df=981.0; Saccharin 2x vs. CTA Retrieval p= 0.0233, q=4.404, df=981.0; Saccharin 2x vs. Extinction p= 0.8426, q=0.8426, df=981.0; Saccharin 2x vs. Reinstatement p= 0.6995, q=0.6995, df=981.0; CTA Retrieval vs. Extinction p= 0.6431, q=2.168, df=981.0; CTA Retrieval vs. Reinstatement p= 0.7735, q=1.868, df= 981.0; Extinction vs. Reinstatement p>0.9999, q=0.3023, df=981.0</p> <p>200pA Saccharin 1x vs. Quinine 1x p= 0.4777, q=2.521, df=981.0; Saccharin 1x vs. Saccharin 2x p= 0.9511, q=1.246, df=981.0; Saccharin 1x vs. CTA Retrieval p= 0.0346, q=4.219, df=981.0; Saccharin 1x vs. Extinction p>0.9999, q=0.07085, df=981.0; Saccharin 1x vs. Reinstatement p= 0.627, q=2.202, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.0862, q=3.75, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.907, q=1.46, df=981.0; Quinine 1x vs. Extinction p= 0.5516, q=2.363, df=981.0; Quinine 1x vs. Reinstatement p>0.9999, q=0.1599, df=981.0; Saccharin 2x vs. CTA Retrieval p= 0.0013, q=5.554, df=981.0; Saccharin 2x vs. Extinction p= 0.9756, q=1.06, df=981.0; Saccharin 2x vs. Reinstatement</p>
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	<p>p= 0.1668, q=3.356, df=981.0; CTA Retrieval vs. Extinction p= 0.0712, q=3.854, df=981.0; CTA Retrieval vs. Reinstatement p= 0.8889, q=1.529, df=981.0; Extinction vs. Reinstatement p= 0.6782, q=2.091, df=981.0</p> <p>250pA Saccharin 1x vs. Quinine 1x p=0.1585, q= 3.389, df=981.0; Saccharin 1x vs. Saccharin 2x p=0.9907, q= 0.8555, df=981.0; Saccharin 1x vs. CTA Retrieval p=0.0038, q= 5.16, df=981.0; Saccharin 1x vs. Extinction p >0.9999, q= 0.3001, df=981.0; Saccharin 1x vs. Reinstatement p= 0.1227, q= 3.547, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.0336, q= 4.233, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.9074, q= 1.459, df=981.0; Quinine 1x vs. Extinction p= 0.1609, q= 3.379, df=981.0; Quinine 1x vs. Reinstatement p= 0.9998, q= 0.3641, df=981.0; Saccharin 2x vs. CTA Retrieval p= 0.0003, q= 6.078, df=981.0; Saccharin 2x vs. Extinction p= 0.9994, q= 0.4763, df=981.0; Saccharin 2x vs. Reinstatement p= 0.0268, q= 4.339, df=981.0; CTA Retrieval vs. Extinction p= 0.0066, q= 4.94, df=981.0; CTA Retrieval vs. Reinstatement p= 0.9838, q= 0.9659, df=981.0; Extinction vs. Reinstatement p= 0.1238, q= 3.541, df=981.0</p> <p>300pA Saccharin 1x vs. Quinine 1x p= 0.0468, q= 4.071, df=981.0; Saccharin 1x vs. Saccharin 2x p= 0.9991, q= 0.5264, df=981.0; Saccharin 1x vs. CTA Retrieval p= 0.0006, q= 5.795, df=981.0; Saccharin 1x vs. Extinction p= 0.9998, q= 0.3808, df=981.0; Saccharin 1x vs. Reinstatement p= 0.023, q= 4.41, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.0153, q= 4.591, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.9311, q= 1.354, df=981.0; Quinine 1x vs. Extinction p= 0.046, q= 4.08, df=981.0; Quinine 1x vs. Reinstatement p= 0.9985, q= 0.585, df=981.0; Saccharin 2x vs. CTA Retrieval</p>
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	<p>p= 0.0001, q= 6.36, df=981.0; Saccharin 2x vs. Extinction p >0.9999, q= 0.09689, df=981.0; Saccharin 2x vs. Reinstatement p= 0.0073, q= 4.898, df=981.0; CTA Retrieval vs. Extinction p= 0.0011, q= 5.594, df=981.0; CTA Retrieval vs. Reinstatement p= 0.9978, q= 0.6315, df=981.0; Extinction vs. Reinstatement p= 0.0229, q= 4.411, df=981.0</p> <p>350pA Saccharin 1x vs. Quinine 1x p= 0.0058, q= 4.992, df=981.0; Saccharin 1x vs. Saccharin 2x p= 0.9998, q= 0.4001, df=981.0; Saccharin 1x vs. CTA Retrieval p= 0.0001, q= 6.284, df=981.0; Saccharin 1x vs. Extinction p >0.9999, q= 0.2699, df=981.0; Saccharin 1x vs. Reinstatement p= 0.0028, q= 5.272, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.0021, q= 5.387, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.9909, q= 0.8504, df=981.0; Quinine 1x vs. Extinction p= 0.0092, q= 4.807, df=981.0; Quinine 1x vs. Reinstatement p= 0.9985, q= 0.5836, df=981.0; Saccharin 2x vs. CTA Retrieval p <0.0001, q= 6.713, df=981.0; Saccharin 2x vs. Extinction p >0.9999, q= 0.09312, df=981.0; Saccharin 2x vs. Reinstatement p= 0.001, q= 5.642, df=981.0; CTA Retrieval vs. Extinction p= 0.0005, q= 5.914, df=981.0; CTA Retrieval vs. Reinstatement p >0.9999, q= 0.1648, df=981.0; Extinction vs. Reinstatement p= 0.0044, q= 5.099, df=981.0</p> <p>400pA Saccharin 1x vs. Quinine 1x p= 0.0062, q= 4.963, df=981.0; Saccharin 1x vs. Saccharin 2x p >0.9999, q= 0.24, df=981.0; Saccharin 1x vs. CTA Retrieval p= 0.0004, q= 5.925, df=981.0; Saccharin 1x vs. Extinction p= 0.9991, q= 0.5192, df=981.0; Saccharin 1x vs. Reinstatement p= 0.0014, q= 5.513, df=981.0; Quinine 1x vs. Saccharin 2x p= 0.0034, q= 5.2, df=981.0; Quinine 1x vs. CTA Retrieval p= 0.9991, q= 0.5284, df=981.0; Quinine 1x vs. Extinction</p>
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		<p>p= 0.0053, q= 5.027, df=981.0; Quinine 1x vs. Reinstatement p= 0.991, q= 0.8492, df=981.0; Saccharin 2x vs. CTA Retrieval p= 0.0002, q= 6.183, df=981.0; Saccharin 2x vs. Extinction p >0.9999, q= 0.3014, df=981.0; Saccharin 2x vs. Reinstatement p= 0.0008, q= 5.736, df=981.0; CTA Retrieval vs. Extinction p= 0.0005, q= 5.857, df=981.0; CTA Retrieval vs. Reinstatement p= 0.9997, q= 0.4195, df=981.0; Extinction vs. Reinstatement p= 0.0013, q= 5.554, df=981.0</p>
Figure 4B	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons fAHP</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p><u>ANOVA results:</u> Treatment; p<0.0001, F (5, 109) =10.64; R squared, 0.3283;</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x p<0.0001, q=9.380, df=109; Saccharin 1x vs. Saccharin 2x p= 0.7249, q= 1.985, df=109; Saccharin 1x vs. CTA Retrieval p=0.0127, q=4.774, df=109; Saccharin 1x vs. Extinction p= 0.9204, q= 1.399, df=109; Saccharin 1x vs. Reinstatement p=0.5239, q= 2.428, df=109; Quinine 1x vs. Saccharin 2x p<0.0001, q= 7.421, df=109; Quinine 1x vs. CTA Retrieval p= 0.0035, q= 5.331, df=109; Quinine 1x vs. Extinction p<0.0001, q= 7.147, df=109; Quinine 1x vs. Reinstatement p= 0.0003, q= 6.299, df=109; Saccharin 2x vs. CTA Retrieval p= 0.4251, q= 2.647, df=109; Saccharin 2x vs. Extinction p= 0.9997, q= 0.4017, df=109; Saccharin 2x vs. Reinstatement p= 0.9983, q= 0.5902, df=109; CTA Retrieval vs. Extinction p= 0.3621, q= 2.796, df=109; CTA Retrieval vs. Reinstatement p= 0.7995, q= 1.799, df=109; Extinction vs. Reinstatement p= 0.9868, q= 0.9191, df=109.</p>
Figure 4C	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons Input Resistance</p> <p>Saccharin 1x Quinine 1x</p>	<p><u>ANOVA results:</u> Treatment; p=0.0213, F (5, 109) =2.775; R squared, 0.1129;</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x p >0.9999, q= 0.03668, df=109; Saccharin 1x vs. Saccharin 2x p= 0.2331, q= 3.152, df=109;</p>

	<p>Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p>Saccharin 1x vs. CTA Retrieval p= 0.9876, q= 0.9065, df=109; Saccharin 1x vs. Extinction p= 0.9997, q= 0.4256, df=109; Saccharin 1x vs. Reinstatement p= 0.3953, q= 2.716, df=109; Quinine 1x vs. Saccharin 2x p= 0.2582, q= 3.075, df=109; Quinine 1x vs. CTA Retrieval p= 0.9859, q= 0.9323, df=109; Quinine 1x vs. Extinction p= 0.9998, q= 0.3877, df=109; Quinine 1x vs. Reinstatement p= 0.4229, q= 2.652, df=109; Saccharin 2x vs. CTA Retrieval p= 0.0352, q= 4.286, df=109; Saccharin 2x vs. Extinction p= 0.5204, q=2.435, df=109; Saccharin 2x vs. Reinstatement p >0.9999, q=0.2024, df=109; CTA Retrieval vs. Extinction p=0.9475, q=1.262, df=109; CTA Retrieval vs. Reinstatement p=0.1001, q=3.711, df=109; Extinction vs. Reinstatement p=0.6757, q= 2.098, df=109.</p>
Figure 4D	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons Sag ratio</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p><u>ANOVA results:</u> Treatment; p=0.0286, F (5, 109) =2.610; R squared, 0.1069;</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x p=0.9862, q= 0.9280, df=109; Saccharin 1x vs. Saccharin 2x p=0.5707, q=2.327, df=109; Saccharin 1x vs. CTA Retrieval p=0.6972, q=2.049, df=109; Saccharin 1x vs. Extinction p= 0.9794, q= 1.015, df=109; Saccharin 1x vs. Reinstatement p=0.9643, q= 1.152, df=109; Quinine 1x vs. Saccharin 2x p=0.2112, q= 3.225, df=109; Quinine 1x vs. CTA Retrieval p= 0.9784, q= 1.026, df=109; Quinine 1x vs. Extinction p>0.9999, q= 0.1598, df=109; Quinine 1x vs. Reinstatement p= 0.7184, q= 2.000, df=109; Saccharin 2x vs. CTA Retrieval p= 0.0209, q= 4.543, df=109; Saccharin 2x vs. Extinction p= 0.2415, q= 3.126, df=109; Saccharin 2x vs. Reinstatement p= 0.9805, q= 1.002, df=109; CTA Retrieval vs. Extinction p= 0.9944, q= 0.7617, df=109; CTA Retrieval vs. Reinstatement</p>

		<p>$p= 0.2504, q= 3.099, df=109;$ Extinction vs. Reinstatement $p= 0.7140, q= 2.010, df=109.$</p>
Figure 4E	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons Action Potential Amplitude</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p><u>ANOVA results:</u> Treatment; $p=0.0054, F(5, 109) = 3.526;$ R squared, 0.1392;</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x $p = 0.2342, q= 3.149, df=109;$ Saccharin 1x vs. Saccharin 2x $p=0.7922, q=1.818, df=109;$ Saccharin 1x vs. CTA Retrieval $p=0.3531, q=2.818, df=109;$ Saccharin 1x vs. Extinction $p= 0.8190, q= 1.746, df=109;$ Saccharin 1x vs. Reinstatement $p=0.9979, q= 0.6222df=109;$ Quinine 1x vs. Saccharin 2x $p=0.0087, q= 4.944, df=109;$ Quinine 1x vs. CTA Retrieval $p= 0.9983, q= 0.5921, df=109;$ Quinine 1x vs. Extinction $p=0.9662, q= 1.137, df=109;$ Quinine 1x vs. Reinstatement $p= 0.5806, q= 2.305, df=109;$ Saccharin 2x vs. CTA Retrieval $p= 0.0129, q= 4.768, df=109;$ Saccharin 2x vs. Extinction $p= 0.1650, q= 3.396, df=109;$ Saccharin 2x vs. Reinstatement $p= 0.5804, q= 2.306, df=109;$ CTA Retrieval vs. Extinction $p= 0.9968, q= 0.6777, df=109;$ CTA Retrieval vs. Reinstatement $p= 0.7511, q= 1.922, df=109;$ Extinction vs. Reinstatement $p= 0.9746, q= 1.065, df=109.$</p>
Figure 4F	<p>One-way ANOVA Kruskal-Wallis test</p> <p>Post-hoc Dunn's multiple comparisons test</p> <p>LIV-VI aIC-BLA neurons Action Potential Half-width</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p><u>ANOVA results:</u> Kruskal-Wallis test; $p=0.0002;$ Kruskal-Wallis statistic, 24.03</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x $p > 0.9999, z= 0.6106$ Saccharin 1x vs. Saccharin 2x $p > 0.9999, z= 0.2586;$ Saccharin 1x vs. CTA Retrieval $p > 0.9999, z= 0.04096;$ Saccharin 1x vs. Extinction $p= 0.0485, z= 2.944;$ Saccharin 1x vs. Reinstatement $p= 0.0200, z= 3.208;$ Quinine 1x vs. Saccharin 2x $p > 0.9999, q= 0.8658;$ Quinine 1x vs. CTA Retrieval $p > 0.9999, z= 0.6129;$ Quinine 1x vs. Extinction</p>

		<p>p= 0.2759, z=2.358; Quinine 1x vs. Reinstatement p=0.1372, z=2.607; Saccharin 2x vs. CTA Retrieval p >0.9999, z=0.3181; Saccharin 2x vs. Extinction p=0.0222, z=3.179; Saccharin 2x vs. Reinstatement p=0.0085, z= 3.448; CTA Retrieval vs. Extinction p=0.0312, z=3.079; CTA Retrieval vs. Reinstatement p=0.0115, z=3.366; Extinction vs. Reinstatement p >0.9999, z= 0.1880.</p>
<p>Figure 4G</p>	<p>One-way ANOVA Post-hoc Tukey's multiple comparisons</p> <p>LIV-VI aIC-BLA neurons Membrane Time Constant</p> <p>Saccharin 1x Quinine 1x Saccharin 2x CTA retrieval Extinction Reinstatement</p>	<p>ANOVA results: Treatment; p= 0.0047, F (5, 109) = 0.1419; R squared,0.1419;</p> <p><u>Multiple Comparisons:</u> Saccharin 1x vs. Quinine 1x p= 0.0987, q= 3.720, df=109; Saccharin 1x vs. Saccharin 2x p= 0.4932, q= 2.495, df=109; Saccharin 1x vs. CTA Retrieval p= 0.1046, q= 3.685, df=109; Saccharin 1x vs. Extinction p=0.9230, q=1.388, df=109; Saccharin 1x vs. Reinstatement p= 0.0022, q=5.525, df=109; Quinine 1x vs. Saccharin 2x p=, 0.9484 q=1.257, df=109; Quinine 1x vs. CTA Retrieval p=0.9999, q=0.3489, df=109; Quinine 1x vs. Extinction p= 0.7139, q=2.010, df=109; Quinine 1x vs. Reinstatement p=0.7124, q=2.014, df=109; Saccharin 2x vs. CTA Retrieval p=0.9798, q=1.011, df=109; Saccharin 2x vs. Extinction p=0.9894, q=0.8761, df=109; Saccharin 2x vs. Reinstatement p=0.2138, q=3.216, df=109; CTA Retrieval vs. Extinction p=0.7866, q=1.833, df=109; CTA Retrieval vs. Reinstatement p=0.4978, q=2.484, df=109; Extinction vs. Reinstatement p=0.0896, q=3.777, df=109.</p>