1 # Intrinsic excitability in layer IV-VI anterior insula to basolateral

2 # amygdala projection neurons encodes the confidence of taste

3 # valence

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# Abstract

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

# Introduction

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

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

79   and palatability (Grossman et al., 2008; Sadacca et al., 2012; Arieli et al., 2020; Vincis et al.,

80   2020). Both synaptic plasticity and neuronal intrinsic properties are proposed to serve as cellular

81   mechanisms underlying learning and memory (Citri and Malenka, 2008; Sehgal et al., 2013).

82   CTA learning promotes LTP induction in the BLA-IC pathway  (Jones et al., 1999; Juárez-

83   Muñoz et al., 2017), and strengthens cell-type specific functional connectivity along the

84   projection (Haley et al., 2016). Intrinsic excitability is the tendency of neurons to fire action

85   potentials when exposed to inputs, reflecting changes in the suit and properties of specific ion

86   channels (Disterhoft et al., 2004; Song and Moyer, 2018). Even though independent mechanisms

87   are involved, recent evidence indicates learning and memory necessitates the that coupling of

88   intrinsic and synaptic plasticity (Turrigiano, 2011; Greenhill et al., 2015; Wu et al., 2021).

89   The IC is an integration hub tuned for the encoding of both exteroceptive as interoceptive

90   information (Gogolla et al., 2014; Haley and Maffei, 2018; Livneh et al., 2020; Koren et al.,

91   2021). By virtue of its extensive network of connectivity, this elongated cortical structure has

92   been shown to integrate sensory, emotional, motivational, and cognitive brain centers through

93   distinct mechanisms. For example, deletions of either *Fos* or *Stk11* in BLA-aIC neurons, alter

94   intrinsic properties at the aIC, and impair CTA acquisition (Levitan et al., 2020). Furthermore,

95   approach behaviors in social decision-making are modulated by subjective and sex-specific

96   affective states that regulate cell-type-specific changes in intrinsic properties at IC terminals

97   receiving hypothalamic input (Rogers-Carter et al., 2018, 2019; Rieger et al., 2022). The

98   posterior IC (pIC) integrates visceral-sensory signals of current physiological states with

99   hypothalamus-gated amygdala anticipatory inputs relating to food or water ingestion, to predict

100  future physiological states (Livneh et al., 2017, 2020). Conversely, aversive visceral stimuli such

101  as LiCl, activate CaMKII neurons projecting to the lateral hypothalamus in right-, but not the left

102 IC, whose optogenetic activation or inhibition can bidirectionally regulate food consumption

103 (Wu et al., 2020). We have previously shown that the aIC-BLA projection is necessary and

104 sufficient for CTA acquisition and retrieval (Lavi et al., 2018; Kayyal et al., 2019), while CTA

105 retrieval requires activation of the projection concomitant with parvalbumin (PV) interneurons

106 (Yiannakas et al., 2021). Moreover, artificial activation of aIC-BLA projecting neurons is

107 sufficient to induce CTA for appetitive taste (Kayyal et al., 2019). Here, using retrograde viral

108 tracing, behavioral analysis, and whole-cell patch-clamp slice electrophysiology, we assessed

109 two hypotheses: (1) That the intrinsic properties of the aIC-BLA projection change as a function

110 of certainty of taste valence prediction, but not percept; and (2) that predictive valence-specific

111 changes in intrinsic properties would be reflected through excitability, being low when taste

112 outcome is highly predictive (i.e., following CTA retrieval or unreinforced familiarization), and

113 high when taste valence is uncertain (i.e., following novelty or extinction). Our data demonstrate

114 for the first time that the intrinsic properties of LIV-VI aIC-BLA neurons are differentially

115 regulated by innate and learned drives, reflecting the confidence of currently perceived taste

116 valence.

117

## Materials and methods

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### Animals

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120 Animals used were 8–12-week-old C57BL/6j (WT) adult male mice. Mice were kept in the local

121 animal resource unit at the University of Haifa on a 12-hour dark/light cycle. Water and chow

122 pellets were available ad libitum, while ambient temperature was tightly regulated. All

123 procedures conducted were approved by the University of Haifa Animal Care and Use

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124  Committee (Ethics License 554/18), as prescribed by the Israeli National Law for the Protection

125  of Animals – Experiments with Animals (1994).

126

127  Animal surgery and viral injections

128  Following surgery and stereotactic injection of viral vectors, behavioral paradigms were

129  performed, as previously described (Yiannakas et al., 2021). Briefly, mice were treated with

130  norocarp (0.5mg/kg), before being anesthetized (M3000 NBT Israel/Scivena Scientific) and

131  transferred to a Model 963 Kopf® stereotactic device. Upon confirming the lack of pain

132  responses, the skull was surgically exposed and drilled to bilaterally inject 0.25μl of

133  ssAAV_retro2-hSyn1-chi-mCherry-WPRE-SV40p(A) (physical titer 8.7 x 10E12 vg/ml), at the

134  BLA (AP -1.58; ML ± 3.375; DV - 4.80). Viral delivery was performed using a Hamilton micro-

135  syringe (0.1uL/minute), while the sculp was cleaned and closed using Vetbond®. Animals were

136  then administered with 0.5mg/kg norocarp and 0.5mg/kg of Baytril (enrofloxacin), and then

137  transferred to a clean and heat-adjusted enclosure for 2 hours. Upon inspection, mice were

138  returned to fresh cages along with similarly treated cage-mates. Weight-adjusted doses of the

139  Norocarp and Baytril were administered for an additional 3 days. All AAV constructs used in this

140  study were obtained from the Viral Vector Facility of the University of Zurich

141  (http://www.vvf.uzh.ch/).

142

143  Electrophysiological studies of the influence of innate taste identity, novelty, and valence

144  on aIC-BLA excitability

145  WT mice treated with viral constructs labeling aIC-BLA projecting neurons were used for

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146   electrophysiological studies. Upon recovery, mice were randomly assigned into treatment groups

147   (Figure 1). Following 24hrs of water deprivation, animals were water restricted for 3 days,

148   receiving water in pipettes ad libitum for 20 minutes/day (Kayyal et al., 2019; Yiannakas et al.,

149   2021). This regime has been extensively used by our lab as it allows rodents to reliably learn to

150   drink from water pipettes with minimal weight loss. Mean total drinking was recorded on the 3$^{rd}$

151   day of water restriction. Novel taste consumption groups were presented with 1.0mL of either

152   0.5% saccharin (*Saccharin 1x*), or Quinine 0.014% (*Quinine 1x*). One hour following the final

153   taste presentation, animals were subjected to patch-clamp electrophysiology (Kayyal et al., 2021;

154   Yiannakas et al., 2021). The *Water* group underwent the same behavioral procedure without

155   novel taste presentations were sacrificed for electrophysiological investigations one hour

156   following water presentation. To dissociate between taste identity and familiarity-related changes

157   in electrophysiological properties, a cohort of mice treated to label the aIC-BLA projection were

158   similarly water deprived following familiarization with saccharin (*Saccharin 5x*). Following the

159   initial water restriction, *Saccharin 5x* animals were allowed access to 0.5% saccharin, in

160   20minute sessions for 4 days. On the fifth day, mice were provided with 1.0ml of the tastant, 1

161   hour prior to sacrifice for electrophysiological recordings. Additionally, WT animals injected

162   with the same viral vector, were allowed a month to recover, following which they were

163   sacrificed for electrophysiological investigations without any behavioral manipulation (*Cage*

164   *Controls*).

165

166   **Electrophysiological studies of the influence of learned aversive taste memory retrieval**

167   **on aIC-BLA excitability**

168   WT mice were treated with viral constructs labelling aIC-BLA projecting neurons to assess the

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169  electrophysiological properties of the projection during aversive or appetitive taste memory

170  retrieval. Upon recovery, mice in CTA retrieval group were trained in CTA for saccharin (LiCl

171  0.14M, 1.5% body weight), while the appetitive saccharin retrieval group (*Saccharin 2x*)

172  received a matching body weight adjusted injection of saline (Yiannakas et al., 2021). Three days

173  following conditioning, both groups underwent a memory retrieval task, receiving 1.0mL of the

174  conditioned tastant 1 hour prior to sacrifice (Figures 2, 4). Brain tissue was extracted and

175  prepared for electrophysiological recording, as above.

176

177  Electrophysiological studies of the influence of learned aversive taste memory extinction

178  and reinstatement on aIC-BLA excitability

179  Electrophysiological studies of CTA extinction and reinstatement were conducted in a cohort of

180  WT male mice (Yiannakas et al., 2021). Following surgery, recovery and water restriction,

181  animals were randomly assigned to the extinction and reinstatement groups (Figures 3-4). Adult

182  male mice used to study extinction and reinstatement were trained in CTA for saccharin

183  following extinction, the reinstatement group received an identical intraperitoneal dose to the

184  original unconditioned stimulus (LiCl 0.14M, 1.5% body weight), 24 hours prior to retrieval.

185  Conversely, the extinction group received a similarly weight-adjusted dose of saline. During the

186  final retrieval session, both groups of mice were allowed access to 1.0mL of the CS, 1 hour prior

187  to sacrifice under deep anesthesia and slice preparation for electrophysiology.

188

189  Electrophysiology tissue preparation

190  The slice electrophysiology and recording parameters were used as described previously (Kayyal

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191   et al., 2021; Yiannakas et al., 2021). Briefly, mice were deeply anesthetized using isoflurane,

192   while brains were extracted following decapitation. Three-hundred um thick coronal brain slices

193   were obtained with a Campden-1000® Vibratome. Slices were cut in ice-cold sucrose-based

194   cutting solution containing the following (in mM): 110 sucrose, 60 NaCl, 3 KCl, 1.25 $NaH_2PO_4$,

195   28 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 5 D-glucose, and 0.6 ascorbate. The slices were allowed to

196   recover for 30 min at 37°C in artificial CSF (ACSF) containing the following (in mM): 125

197   NaCl, 2.5 KCl, 1.25 $NaH_2PO_4$, 25 $NaHCO_3$, 25 D-glucose, 2 $CaCl_2$, and 1 $MgCl_2$. Slices were

198   then kept for an additional 30 min in ACSF at room temperature until electrophysiological

199   recording. The solutions were constantly gassed with carbogen (95% $O_2$, 5% $CO_2$).

200

201   Intracellular whole cell recording

202   After the recovery period, slices were placed in the recording chamber and maintained at 32-

203   34°C with continuous perfusion of carbogenated ACSF (2 ml/min). Brain slices containing the

204   anterior insular cortices were illuminated with infrared light and pyramidal cells were visualized

205   under a differential interference contrast microscope with 10X or 40X water-immersion

206   objectives mounted on a fixed-stage microscope (BX51-WI; Olympus®). The image was

207   displayed on a video monitor using a charge-coupled device (CCD) camera (QImaging®,

208   Canada). Insula to BLA projection cells infected with AAV were identified by visualizing

209   mCherry^{+} cells. Recordings were amplified by Multiclamp™ Axopatch™ 200B amplifiers and

210   digitized with Digidata® 1440 (Molecular Devices®). The recording electrode was pulled from a

211   borosilicate glass pipette (3–5 M) using an electrode puller (P-1000; Sutter Instruments®) and

212   filled with a K-gluconate-based internal solution containing the following (in mM): 130 K-

213   gluconate, 5 KCl, 10 HEPES, 2.5 $MgCl_2$, 0.6 EGTA, 4 Mg-ATP, 0.4 Na3GTP and 10

214   phosphocreatine (Na salt). The osmolarity was 290 mOsm, and pH was 7.3. The recording glass

215   pipettes were patched onto the soma region of mCherry^{+} pyramidal neurons and neighboring non

216   fluorescent pyramidal neurons.

217   The recordings were made from the soma of insula pyramidal cells, particularly from layer 2/3

218   and Layer 5/6. Liquid junction potential (10 mV) was not corrected online. All current clamp

219   recordings were low pass filtered at 10 kHz and sampled at 50 kHz. Pipette capacitance and

220   series resistance were compensated and only cells with series resistance smaller than 20 MΩ

221   were included in the dataset. The method for measuring active intrinsic properties was based on a

222   modified version of previous protocols (Kaphzan et al., 2013; Chakraborty et al., 2017; Sharma

223   et al., 2018).

224

225   Recording parameters

226   Resting membrane potential (RMP) was measured 10 sec after the beginning of whole cell

227   recording (rupture of the membrane under the recording pipette). The dependence of firing rate

228   on the injected current was obtained by injection of current steps (of 500ms duration from 0 to

229   400 pA in 50 pA increments). Input resistance was calculated from the voltage response to a

230   hyperpolarizing current pulse (-150 pA). SAG ratio was calculated from voltage response -150

231   pA. The SAG ratio during the hyperpolarizing steps was calculated as $[(1-\Delta V_{SS}/ \Delta V_{max})$ x

232   100%] as previously reported by (Song, Ehlers, & Moyer, 2015). The membrane time constant

233   was determined using a single exponential fit in the first 100ms of the raising phase of cell

234   response to a 1 second, -150 pA hyperpolarization step.

235   For measurements of a single action potential (AP), after initial assessment of the current

236   required to induce an AP at 15ms from the start of the current injection with large steps (50 pA),

237   a series of brief depolarizing currents were injected for 10ms in steps of 10 pA increments. The

238   first AP that appeared on the 5ms time point was analyzed. A curve of dV/dt was created for that

239   trace and the 30 V/s point in the rising slope of the AP was considered as threshold (Chakraborty

240   et al., 2017). AP amplitude was measured from the equipotential point of the threshold to the

241   spike peak, whereas AP duration was measured at the point of half-amplitude of the spike. The

242   medium after-hyperpolarization (mAHP) was measured using prolonged (3 seconds), high-

243   amplitude (3 nA) somatic current injections to initiate time-locked AP trains of 50 Hz frequency

244   and duration (10 –50 Hz, 1 or 3 s) in pyramidal cells. These AP trains generated prolonged (20 s)

245   AHPs, the amplitudes and integrals of which increased with the number of APs in the spike train.

246   AHP was measured from the equipotential point of the threshold to the anti-peak of the same

247   spike (Gulledge et al., 2013). Fast (fAHP), and slow AHP (sAHP) measurements were identified

248   as previously described (Andrade et al., 2012; Song and Moyer, 2018). Series resistance, Rin,

249   and membrane capacitance were monitored during the entire experiment. Changes of at least

250   30% in these parameters were criteria for exclusion of data.

251   Classification of Burst and Regular spiking neurons

252   At the end of recordings, neurons were classified as either burst (BS) or regular spiking (RS) as

253   reported previously (Kim et al., 2015; Song et al., 2015). Briefly, neurons that fired two or more

254   action potentials (doublets or triplets) potential towards a depolarizing current step above the

255   spike threshold current were defined as burst spiking (BS). Regular spiking (RS) neurons on the

256   other hand, were defined as neurons that fired single action potential in response to a

257   depolarizing current step above spike threshold (Extended Figure 1-2A).

258

259    Statistical analysis of individual intrinsic properties across treatments

260    Individual intrinsic properties of aIC-BLA projecting neurons in the respective treatment groups

261    (Figures 1-4) were analyzed using appropriate statistical tests (One-way or Two-way ANOVA,

262    GraphPad Prism®), as defined in the Statistics Table. Two-way repeated measurements of

263    analysis of variance (RM-ANOVA) followed by Sidak's (for two groups) or Tukey's (for more

264    than two groups) post-hoc multiple comparison test was performed for firing properties. The

265    intrinsic properties were determined with Two-tailed unpaired t-tests, and One-way ANOVA

266    followed by Tukey's or Dunn's multiple comparisons test were used. For all tests, $*p < 0.05$ was

267    considered significant. Following spike-sorting, the ratio of BS:RS aIC-BLA projecting neurons

268    in the sampled population was compared across our treatments (Mann-Whitney test, GraphPad

269    Prism®). Similarly, individual intrinsic properties in BS and RS aIC-BLA projecting neurons

270    were analyzed following spike-sorting (One-way or Two-way ANOVA, GraphPad Prism®). All

271    data reported as mean ± standard error (SEM).

272

273    Immunohistochemistry

274    From each electrophysiological recording, three 300μm-thick mouse brain slices were obtained

275    starting from Bregma coordinates 1.78, 1.54 and 1.18, respectively. Slices were washed with

276    PBS and fixed using 4% paraformaldehyde in PBS at $4^0$C for 24 hours. Slices were then

277    transferred to 30% sucrose/PBS solution for 48 hours and mounted on glass slides using

278    Vectashield® mounting medium with DAPI (H-1200). Slides were then visualized using a

279    vertical light microscope at 10x and 20x magnification (Olympus CellSens Dimension®).

280    Images were processed using Image-Pro Plus® V-7 (Media Cybernetics). The localization of

281    labelled mCherry+ neurons in the agranular aIC - where recordings were obtained from, was

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282    quantified manually across three Bregma-matched slices, for each animal. Quantification was

283    done using randomly assigned IDs for individual animals, regardless of treatment.

284    Representative images were additionally processed using the Olympus CellSens 2-D

285    deconvolution® function.

286

287    Principal component analysis (PCA) of the profile of intrinsic properties across treatment

288    groups

289    Principal component analysis (PCA) of the standardized intrinsic properties of the LIV-VI aIC-

290    BLA (Figure 5; Extended Figure 5-1) was performed using the correlation matrix on GraphPad

291    Prism9, MATLAB R2020b, and IBM SPSS Statistics 27. The covariance matrix was used for

292    each PCA was performed in six behavioral groups, the low memory prediction (Saccharin 1x,

293    n=20; Saccharin 2x, n=20, and Extinction, n=14), and the high memory prediction (Saccharin 5x,

294    n=18; CTA retrieval, n=27, and Reinstatement, n=15), RS vs. BS neurons. A total of 114 neurons

295    (BS vs. RS) across all intrinsic properties and excitability changes (50–400 pA) (Extended

296    Figure 5-1A), and later all intrinsic properties with only 350 pA (highest excitability differences

297    between treatment groups; Extended Figure 5-1, B). PCA was conducted on 63 burst spiking

298    neurons using 12 variables: 350 (pA), RMP (mV), mAHP (mV), sAHP (mV), fAHP (mV), IR

299    (MΩ), Sag Ratio, Time constants (msec), AP amplitude (mV), AP Halfwidth (msec), AP

300    threshold (mV), Rheobase (pA), (Figure 5 A &B). The adequacy of the sample was evaluated

301    using the Bartlett's test and the Kaiser-Meyer-Olkin (KMO) measure was applied. The degrees

302    of freedom (df) were calculated using the following formula:

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

303    The number of principal components was chosen according to the percentage of variance

304    explained (>75%). The parallel analysis evaluated the optimal number of components and

305    selected 3 PCs, explaining 62.47% of the variance. Oblique factor rotation (par) of the first three

306    PCA components, using a standard 'rotatefactors' routine from MATLAB Statistics Toolbox.

307    This approach maximizes the varimax criterion using an orthogonal rotation. To optimize

308    variance, oblique factor rotation (paramax) was used, and the threshold chosen to define a

309    variable as a significant contributor was a variance $\geq 0.7$ given the small sample size. The

310    correlation matrix was adequate as the null hypothesis of all zero correlation was rejected

311    [$\chi_{66}^2$=387.444, p<0.001], and KMO exceeded 0.5 (KMO=0.580).

312    To calculate the proportion of the variance of each variable that the principal components can

313    explain, communalities were calculated and ranged from 0.426 to 0.897 (extended Figure 5-1, -A

314    C). The communalities scores were calculated using the following formula: $= \frac{\sum_{i=1}^{m}\lambda}{\#variables*\lambda}$; where

315    *m* is the number of selected PCs. The threshold chosen was Comm $\geq 60\%$.

316    Due to the imbalance in sample sizes between groups, the PCA space is biased in favor of the

317    group with bigger sample size. The BS neurons in the six behavioral groups previously men-

318    tioned were resampled to ensure that sample sizes were balanced across groups datasets (Figure

319    5, A&B). Particularly, we reduced the number of Saccharin 1X and 2X observations by using

320    random sampling ("randn" function in MATLAB); for Saccharin 1x, we chose 10 of the 17 total

321    elements, and for Saccharin 2x, we selected 10 of the 13 total elements.

322    **K-means clustering**

323    To assess the distribution of bursting neurons in multidimensional space, we performed a k-

324    means cluster analysis in MATLAB (k = 2 clusters, maximum iterations was 100 with random

325    starting locations, squared Euclidean distance metric used) for the principal components, which

326    explained 62.47% of the variance in intrinsic properties (Figure 5; extended Figure 5-1 and 5-2).

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327

## Results

329 To prove or refute our hypotheses, we conducted a series of electrophysiological recordings in

330 slice preparation from the mouse aIC, in which we labelled aIC-BLA projecting neurons using

331 retrograde adeno-associated viral tracing – retro AAV (see Methods). Electrophysiological

332 recordings were obtained from the aIC-BLA projecting neurons in LI-III (Table 2) and LIV-VI

333 (Table 1), following novel appetitive or aversive taste stimuli (Figure 1), following appetitive or

334 aversive taste memory retrieval (Figure 2), as well as following extinction and reinstatement

335 (Figures 3-4). We focused on deep-lying LIV-VI aIC-BLA projecting neurons (Table 1), as

336 intrinsic properties in superficial LI-III aIC-BLA projecting neurons were unaffected by taste

337 identity, familiarity, or valence (Table 2). We measured action potential (AP) firing frequency in

338 response to incrementally increasing depolarizing current injections, as well as 11 distinct of

339 intrinsic properties (Tables 1-2 and Statistics Table): resting membrane potential (RMP); slow,

340 medium and fast after-hyperpolarization (sAHP, mAHP, fAHP); input resistance (IR), SAG

341 ratio; the amplitude, half-width and threshold for APs; the time taken for a change in potential to

342 reach 63% of its final value (membrane time constant - $\tau$), as well as the minimum current

343 necessary for AP generation (Rheobase). Statistical analysis was conducted using repeated

344 measures one- or two-way ANOVA (see Statistics table).

345

346 **When taste is both appetitive and novel, excitability in LIV-VI aIC-BLA projection**

347 **neurons is increased**

348 To delineate the mechanisms through which novelty is encoded on the LIV/VI aIC-BLA

17

349 projection, we labeled the projection (see methods), and compared the intrinsic properties across

350 neutral, innately aversive, and innately appetitive taste stimuli. Following surgery recovery mice

351 were randomly assigned to the following behavioral groups: Water (Control for procedure,

352 Water; n=6 animals, 23 cells), 0.5 % Saccharin for the first (Novel innate appetitive, Saccharin

353 1x; n=5 animals, 20 cells), or fifth time (Familiar appetitive, Saccharin 5x; n=6 animals, 18

354 cells), 0.04% Quinine (Novel aversive, Quinine 1x; n=4 animals, 19 cells), or cage controls that

355 did not undergo water-restriction (Base-line control, Cage control; n=4 animals, 19 cells). This

356 approach allowed us to examine excitability changes that relate to the innate aversive/appetitive

357 nature and novelty/familiarity associated with tastants, while accounting for the effects of acute

358 drinking, as well as the water restriction regime itself. Guided by evidence regarding the

359 induction of plasticity cascades, the expression of immediate early genes, as well as the

360 timeframes involved in LTP and LTD at the IC (Rosenblum et al., 1997; Hanamori et al., 1998;

361 Jones et al., 1999; Escobar and Bermúdez-Rattoni, 2000), the five treatment groups were

362 sacrificed 1 hour following taste consumption. Even though changes in activity can be observed

363 within seconds to minutes, depending on their novelty, salience and valence (Barot et al., 2008;

364 Lavi et al., 2018; Wu et al., 2020), sensory experiences can modulate the function of IC neurons

365 for hours (Juárez-Muñoz et al., 2017; Rodríguez-Durán et al., 2017; Haley et al., 2020; Kayyal et

366 al., 2021; Yiannakas et al., 2021).We had previously identified a CaMKII-dependent short-term

367 memory trace at the IC that last for the first 3 hours following taste experiences, regardless of

368 their valence (Adaikkan and Rosenblum, 2015). To address whether similar time-dependency of

369 the physiological correlations engaged by the IC during novel taste learning, a 6th group was

370 sacrificed 4 hours following novel saccharin exposure (Figure 1 – Saccharin 1x (4hrs)).

371 Daily water intake prior to the final taste exposure and the acquisition of electrophysiological

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372     data was not different among the five groups that underwent water restriction (Figure 1B, One-

373     way ANOVA, p=0.4424, F =0.9766, R squared =0.1634). However, excitability in response to

374     incremental depolarizing currents was significantly different between the six groups (Figure 1D

375     – Two-way ANOVA, p<0.0001, F (8,880) =1269). Exposure to saccharin for the first time (i.e.,

376     novel appetitive), at the 1-hour time-point, resulted in enhanced excitability on the aIC-BLA

377     projection compared to all other groups (Figure 1D, see Table 1). Conversely, fAHP (Figure 1H;

378     One-way ANOVA, p<0.0001, F =8.380, R squared =0.2758) in the Quinine 1x group was

379     increased compared to all other groups, in contrast to Saccharin 1x where it was most suppressed

380     (see Table 1). In fact, fAHP in the Saccharin groups recorded at 1hr (p<0.0001, z =5.150) or

381     4hours (p=0.0099, z =3.406) following novel taste consumption was suppressed compared to

382     innately aversive Quinine 1x (Figure 1H). Even though fAHP in the Saccharin 1x group was

383     suppressed compared to both the Cage control (p=0.0136, z =3.318) and Water (p=0.0177, z

384     =3.243) groups, this was not the case for the Saccharin 1x (4hr) group (p>0.9999 for both – see

385     Table 1). Importantly, fAHP (Figure 1H) was nearly identical in treatment groups where the

386     tastant could be deemed as highly familiar and safe, such as the Cage control group (that did not

387     undergo water restriction), as well as animals in the Water or Saccharin 5x groups (that had

388     undergone water restriction).

389     Significant differences in terms of τ (Figure 1J; Kruskal-Wallis test, p=0.0005, Kruskal-Wallis

390     statistic=21.91), were only observed between the Cage control and Saccharin 1x (4hrs) groups

391     (p=0.0040, z=3.647), as well as the Saccharin 1x and Saccharin 1x (4hrs) groups (p=0.0029,

392     z=3.725). On the other hand, significant differences in AP half-width (Figure 1I; Kruskal-Wallis

393     test; p=0.0125, Kruskal-Wallis statistic=14.54) were only observed between the Saccharin 1x

394     (4hrs) compared to Saccharin 1x (p=0.0065, z =3.519) groups (see Table 1).

19

395 These results demonstrate that in the context of taste novelty, innately appetitive saccharin drove

396 increases in excitability and decreases in fAHP of LIV-VI aIC-BLA projecting neurons,

397 compared to innately aversive quinine (Figure 1D, H). Compared to the Cage control and Water

398 groups, fAHP on the projection was significantly enhanced by innately aversive quinine and was

399 suppressed by innately appetitive novel saccharin (Figure 1H). However, the effect of appetitive

400 taste novelty on firing frequency was time-dependent, as it was observed at 1hr, but not 4hrs

401 following novel taste exposure (Figure 1D). Furthermore, following familiarity acquisition for

402 saccharin (Saccharin 5x), excitability was suppressed compared to Saccharin 1x, matching the

403 Cage control, Water, and Quinine 1x groups (Figure 1D). This led us to consider whether

404 increased excitability is not related to taste identity or palatability (Wang et al., 2018), but the

405 perceived salience of taste experiences, which encompasses both novelty and valence (Ventura et

406 al., 2007; Kargl et al., 2020). Previous studies have suggested that the induction of plasticity

407 signaling cascades and IEGs in pyramidal neurons of the aIC (commonly used as surrogates for

408 changes in excitability), is a crucial step for the association of taste and visceral information

409 during CTA learning (Adaikkan and Rosenblum, 2015; Soto et al., 2017; Wu et al., 2020).

410 Activation of the aIC-BLA projection is indeed necessary for the expression of neophobia

411 towards saccharin (Kayyal et al., 2021), as well as for CTA learning and retrieval (Kayyal et al.,

412 2019). Yet, its chemogenetic inhibition does not affect the attenuation of neophobia, nor the

413 expression of aversion towards innately aversive quinine (Kayyal et al., 2019). Furthermore,

414 aversive taste memory retrieval necessitates increases in pre-synaptic inhibitory input on the

415 projection (Yiannakas et al., 2021). Bearing this in mind, we hypothesized that increases in

416 excitability on the projection could be indicative of a labile state of the taste trace at the aIC,

417 which manifests when taste cues are not (yet) highly predictive of the visceral outcome of the

20

418   sensory experience (Bekisz et al., 2010; Galliano et al., 2021). In such a scenario, taste memory

419   retrieval following strong single-trial aversive learning would be expected to result in decreased

420   excitability compared to control animals. To assess this hypothesis, we next examined intrinsic

421   excitability in mice retrieving an appetitive (Saccharin 2x, CTA retrieval control) or learned

422   aversive memory (CTA retrieval) for saccharin.

423

424   **Learned aversive taste memory retrieval suppresses the excitability of LIV-VI aIC-BLA**

425   **projecting neurons**

426   Following recovery from rAAV injection, mice in the CTA retrieval group underwent water

427   restriction and CTA conditioning for 0.5% saccharin (see Methods, Figure 2A).

428   Electrophysiological recordings were obtained from aIC-BLA neurons 3 days later, 1 hour

429   following retrieval (n=8 animals, 27 cells). Mice in the Saccharin 2x group on the other hand,

430   were familiarized with saccharin without conditioning, and recordings were obtained within the

431   same period, following retrieval (n=5 animals, 20 cells). Through this approach we aimed to

432   examine the hypothesis that like innately aversive and highly familiar appetitive responses

433   (Figure 1), learned aversive taste memory retrieval would be correlated with suppression of the

434   intrinsic excitability on the projection.

435   As expected, CTA retrieval mice, exhibited suppressed consumption of the conditioned tastant

436   compared to control animals that were only familiarized with saccharin (Figure 2B, Mann-

437   Whitney test, p=0.0085; Sum of ranks: 52.50, 38.50; Mann-Whitney U =2.500). Intrinsic

438   excitability in LIV-VI aIC-BLA projecting neurons was increased in response to depolarizing

439   current injections (Figure 2D; $p<0.0001$, $F_{(8, 360)} =483.3$), and was significantly different

440   between the two treatments (p=0.0014, F (1, 45) =11.60). Excitability was enhanced in the

441   Saccharin 2x group compared to CTA retrieval, while a significant interaction was identified

442   between the treatment and current injection factors (p<0.0001, F (8, 360) =9.398). Fast AHP

443   (fAHP) on LIV-VI aIC-BLA projecting neurons tended to be increased in the CTA retrieval

444   group (see Table 1), however differences compared to Saccharin 2x failed to reach significance

445   (Unpaired t-test; p=0.0527, t=1.990, df=45). Conversely, AP amplitude in the Saccharin 2x group

446   was significantly decreased compared to CTA retrieval (Figure 2G; Unpaired t-test; p=0.0002,

447   t=3.983, df=45). In addition, the CTA retrieval group exhibited significantly decreased IR

448   (Figure 2H; Unpaired t-test; p=0.0036, t=3.072, df=45) and significantly enhanced SAG ratio

449   (Figure 2I; Unpaired t-test; p=0.0037, t=3.060, df=45), compared to Saccharin 2x. In accord with

450   our hypothesis, excitability on LIV-VI aIC-BLA projecting neurons was suppressed by aversive

451   taste memory retrieval. We have previously shown that compared to CTA retrieval and

452   reinstatement, appetitive memory retrieval and extinction were associated with (a) an

453   enhancement of IEG induction (c-fos and Npas4) at the aIC, and (b) decreased frequency of pre-

454   synaptic inhibition on the aIC-BLA (Yiannakas et al., 2021). In accord, other published work

455   investigating the induction of IEG in the rodent IC, found that consistent with a reduction in

456   spiking activity (Grossman et al., 2008), the induction of c-fos at the IC was suppressed by

457   aversive taste memory retrieval (Haley et al., 2020). Earlier studies have also reported increases

458   in c-fos following the extinction of cyclosporine A-induced CTA (Hadamitzky et al., 2015). We

459   thus hypothesized that if excitability in these cells serves as key node for a change in valence

460   prediction, extinction - which constitutes a form of appetitive re-learning, would be associated

461   with enhanced excitability compared to CTA retrieval and reinstatement (Berman, 2003; Suzuki

462   et al., 2004; Morrison et al., 2016; Slouzkey and Maroun, 2016). In addition, through these

463    extinction and reinstatement studies, we were able to examine the real-life relevance of these

464    changes on intrinsic excitability, in a context where behavioral performance reflects the balance

465    between contrasting memories and the availability of retrieval cues (Figure 3).

466

467    **The predictability of the valence arising from taste experiences determines the profile of**

468    **intrinsic properties of LIV-VI aIC-BLA projecting neurons**

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

470    projecting neurons from mice having undergone unreinforced CTA extinction (Extinction; n=5,

471    14 cells), or US-mediated CTA reinstatement (Reinstatement; n=3 animals, 15 cells).

472    Behaviorally, the two groups of animals were similar in terms of their aversion profile over 9

473    unreinforced extinction sessions (Figure 3B; 2-way ANOVA; Extinction: $p<0.0001$, $F_{(8, 54)}$

474    $=13.44$; Treatment: $p=0.0681$, $F_{(1, 54)} =3.466$; Interaction: $p=0.9697$, $F_{(8, 54)} =0.2803$). As

475    expected, saccharin consumption during the test day in the Reinstatement group was decreased

476    compared to Extinction (Figure 3C; Mann-Whitney test; $p=0.0179$; Sum or ranks: 30, 6; Mann-

477    Whitney U=0). Consistent with our findings in Figure 2, aversive taste memory retrieval in the

478    Reinstatement group was associated with suppressed excitability compared to the Extinction

479    group (Figure 3E; 2-way ANOVA, Current injection: $p<0.0001$, $F_{(8, 216)} =370.1$; Treatment:

480    $p=0.0297$, $F_{(1, 27)} =5.291$; Interaction: $p<0.0001$, $F_{(8, 216)} =10.30$). CTA Reinstatement was

481    also associated with increases in the AP threshold (Figure 3F; Unpaired t-test: $p=0.0076$, t

482    $=2.887$, df=27) and $\tau$ (Figure 3G; Unpaired t-test: $p=0.0153$, $t=2.589$, df=27) compared to

483    Extinction.

484    Unlike animals that underwent familiarization with the tastant without conditioning (Figure 1),

23

485    excitability on the projection in the Extinction group was not suppressed by familiarization

486    (Figure 3). Conversely, even though the intrinsic mechanisms employed would appear to differ,

487    aversive taste memory retrieval regardless of prior experience, was associated with baseline

488    excitability of the aIC-BLA projection (Figure 3). Our findings in this section (Figure 3),

489    revealed that during taste memory retrieval, excitability on the projection is not solely dependent

490    on the relevant novelty or appetitive nature of tastants, and does not subserve the persistence of

491    CTA memories (Figure 2). Instead, excitability on the aIC-BLA projection is indeed shaped by

492    prior experience but is best predicted by the probability for further aversive (re)learning.

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

494    familiarity, and valence over the course of time and experience, we compared the profile of

495    intrinsic properties across pairs of behavioral groups in which the currently perceived novelty, as

496    well as innate or learned valence associated with taste was notably different. Through this

497    comparison, we were led to conclude that excitability on aIC-BLA projecting neurons is driven

498    by taste stimuli of positive valence, however this effect is dependent on subjective experience

499    and the possibility for further associative learning (Figure 4A). Excitability on aIC-BLA

500    projecting neurons in the treatment groups where the tastant was perceived as appetitive

501    (Saccharin 1x, Saccharin 2x and Extinction), was closely matched, and was significantly

502    enhanced compared to the innately or learned aversive (Quinine 1x, CTA retrieval and

503    Reinstatement) groups (Figure 4A; Two-way ANOVA; Current injection: $p<0.0001$, $F_{(5, 872)}$

504    $=1218$; Treatment: $p=0.0014$, $F_{(5, 109)} =4.281$; Interaction: $p<0.0001$, $F_{(40, 872)} =4.978$). As

505    previously identified in Figure 1H, fAHP reflected the innate aversive nature of the tastant, being

506    increased in the Quinine 1x group compared to all other groups (Figure 4B; One-way ANOVA;

507    $F =10.65$, $p<0.0001$, R squared $=0.3283$, see Table 1). Significant differences in IR (Figure 4C;

24

508  One-way ANOVA; F = 2.775, p=0.0213, R squared =0.1129) and SAG ratio (Figure 4D; One-

509  way ANOVA; F =2.610, p=0.0286, R squared =0.1069) were only observed between the CTA

510  retrieval and Saccharin 2x groups. AP amplitude (Figure 4E; One-way ANOVA, p=0.0054,

511  F=3.526, R squared =0.1392) in the Saccharin 2x group was suppressed compared to both CTA

512  retrieval (p=0.0129, q=4.768, df=109) and Quinine 1x (p=0.0087, q=4.944, df=109). Conversely,

513  the Extinction and Reinstatement groups, where familiarity with the tastant was the highest,

514  exhibited increased AP half-width compared to all other groups (Figure 4F; One-way ANOVA,

515  Kruskal-Wallis test; p=0.0002; Kruskal-Wallis statistic, 24.03). Significant differences in terms

516  of τ (Figure 4G; One-way ANOVA, p=0.0047, F =3.606) were only observed in comparing the

517  Saccharin 1x and Reinstatement groups (p=0.0022, q=5.525, df=109). Hence, neuronal

518  excitability is indeed a feature associated with predictive power to modulate taste valence,

519  however it does not fully reflect the breadth of intrinsic property changes among the different

520  behavioral groups.

521

522  The predictability of taste valence intrinsic is primarily reflected on the excitability of

523  burst-spiking, but not regular-spiking LIV-VI aIC-BLA projecting neurons

524  Our initial analysis of individual intrinsic properties (Figures 1-3) highlighted that excitability is

525  enhanced following appetitive experiences in which the internal representation is still labile and

526  is associated with the possibility for further aversive learning (novelty or extinction). Conversely,

527  following extensive familiarization, aversive conditioning, or reinstatement, whereby taste

528  exposure leads to memory retrieval of specific valence, excitability on LIV-VI aIC-BLA

529  projecting neurons was similar to baseline (Figures 1 and 4). While the precise mechanism

530  through which sensory input is encoded at the cortex (and other key regions), is still a matter of

25

531 ongoing research, studies indicate that bursting in cortical layer V pyramidal neurons can encode

532 oscillating currents into a pattern that can be reliably transmitted to distant post-synaptic

533 terminals (Kepecs and Lisman, 2003; Samengo et al., 2013; Zeldenrust et al., 2018). Spike burst

534 is defined as the occurrence of three or more spikes from a single neurons with <8ms intervals

535 (Ranck, 1973; Connors et al., 1982). In brain slices from naïve mice, half of the neurons of a

536 given structure exhibit burst firing, while the distribution of burst-spiking (BS) to regular-spiking

537 (RS) neurons, changes along the anterior-posterior axis of the subiculum (Staff et al., 2000;

538 Jarsky et al., 2008). Importantly, the two cell types fine-tune the output of brain structures by

539 virtue of differences in synaptic plasticity, as well as intrinsic excitability mechanisms (Graves et

540 al., 2012; Song et al., 2012). Furthermore, there are changes in the ratio of BS:RS neurons in

541 individual brain structures, as well as differences in the recruitment of signaling events, ion

542 channels and metabotropic receptors among the two cell types (Wozny et al., 2008; Shor et al.,

543 2009). Correspondingly, complex region and task-specific rules govern the molecular and

544 electrophysiological mechanisms through which information encoding and retrieval takes place

545 in the two cell types (Dunn et al., 2018; Dunn and Kaczorowski, 2019). Little is currently known

546 regarding the influence of cell identity in the repertoire of plasticity mechanisms employed by

547 the IC to facilitate taste-guided behaviors (Maffei et al., 2012; Haley and Maffei, 2018).

548 Our post-hoc spike sorting analysis allowed us to distinguish between BS and RS LIV-VI aIC-

549 BLA projecting neurons, and thus their relative contribution to behaviorally driven changes in

550 the suit of intrinsic properties (Extended Figures 1-3, 2-1 and 3-1). Through this comparison, we

551 uncovered that Saccharin 1x differed to other groups in terms of excitability and fAHP in BS

552 LIV-VI aIC-BLA neurons (Extended Figure 1-3), while no such changes were observed in RS

553 neurons (see Summary of RS intrinsic properties table no.4). Similarly, excitability in the

26

554   Saccharin 2x group was significantly enhanced compared to CTA retrieval in BS-, but not in RS

555   LIV-VI aIC-BLA neurons (Extended Figure 2-1A, F). Significant differences in IR, SAG ratio

556   and AP amplitude between CTA retrieval and Saccharin 2x were primarily driven by BS LIV-VI

557   aIC-BLA neurons (Extended Figure 2-1B-D, G-I). Conversely, significant differences in AP half-

558   width between the aversive and appetitive memory retrieval groups were only observed in RS

559   neurons (Extended Figure 2-1J). Correspondingly, excitability in the Extinction group was

560   enhanced compared to Reinstatement in BS-, and not RS LIV-VI aIC-BLA neurons (Extended

561   Figure 3-1A, H). Indeed, excitability on BS LIV-VI aIC-BLA neurons following extinction and

562   reinstatement, reflected the subjective predictability of taste memory retrieval, being high

563   following extinction compared to reinstatement (Extended Figure 3-1). However, this effect was

564   mediated through alternative mechanisms compared to single-trial learning and memory retrieval

565   (Extended Figure 1-3, 2-1, 3-1). Significant differences between the Extinction and

566   Reinstatement groups, were observed in terms of the sAHP, AP threshold, SAG ratio and $\tau$ in BS

567   but not in RS LIV-VI aIC-BLA neurons (Extended Figure 3-1B-F).

568   Encouraged by these findings, we focused on the Saccharin 1x, Saccharin 2x, Saccharin 5x, CTA

569   Retrieval, Extinction and Reinstatement groups, as to isolate the contribution of BS LIV-VI aIC-

570   BLA neurons in encoding the subjective predictability of taste experience during taste learning,

571   re-learning, and memory retrieval (Figure 5). Consistent with studies in the hippocampus

572   (Graves et al., 2016), we found that the percentage of BS LIV-VI aIC-BLA projecting neurons in

573   the sampled population was highest in the context of novel taste learning (Extended Figure 1-2B:

574   Saccharin 1x, 85%), and subsided following progressive familiarization (Extended Figure 1-2B;

575   Saccharin 2x, 65%, Mann-Whitney test: p=0.0562; Sum of ranks: 303.5, 161.5; Mann-Whitney

576   U =70.50; Saccharin 5x, 55.56%, Mann-Whitney test: p=0.0034; Sum of ranks: 291, 87; Mann-

577    Whitney U =32;). Interestingly, animals retrieving CTA, exhibited the lowest proportion of BS

578    neurons among the six treatments (Extended Figure 1-2B; CTA retrieval, 44.44% BS), and

579    significant differences were observed compared to control animals (Extended Figure 1-2B;

580    Saccharin 2x, 65% BS; Mann-Whitney test: p=0.0102; Sum of ranks: 257, 208; Mann-Whitney

581    U =55). Thus, the ratio of BS:RS LIV-VI aIC-BLA projecting neurons was plastic in relation to

582    experience and was highest in response to appetitive novelty – in accord with studies

583    investigating the intrinsic excitability of subiculum output neurons in relation to contextual

584    novelty and valence encoding (Dunn et al., 2018). Indeed, the ratio of BS:RS LIV-VI aIC-BLA

585    neurons was progressively suppressed by familiarity acquisition (Saccharin 1x> 2x> 5x), as well

586    as following aversive taste memory recall (CTA retrieval), compared to both appetitive learning

587    (Extended Figure 1-2B; Saccharin 1x, Mann-Whitney test: p<0.0001; Sum of ranks: 407, 188;

588    Mann-Whitney U =35) and re-learning (Extended Figure 1-2B; Extinction, Mann-Whitney test:

589    p=0.0007; Sum of ranks: 182, 224; Mann-Whitney U =29). However, our comparison failed to

590    account for the influence of complex experiences over time, as differences between Extinction

591    and Reinstatement failed to reach significance (Extended Figure 1-2B; Mann-Whitney test:

592    p=0.3870, Sum of ranks: 133.5, 97.50, Mann-Whitney U =42.50), while perplexingly, the ratio

593    of BS:RS aIC-BLA neurons in these groups was differentially increased compared to Saccharin

594    5x (Extended Figure 1-2B; Extinction, Mann-Whitney test: p=0.0300; Sum of ranks: 81, 150;

595    Mann-Whitney U =26; Reinstatement, Mann-Whitney test: p=0.3498; Sum of ranks: 90, 120;

596    Mann-Whitney U =35;), but not Saccharin 2x (Extended Figure 1-2B; Extinction, Mann-

597    Whitney test: p=0.2397; Sum of ranks: 143.5, 156.5; Mann-Whitney U =52.50; Reinstatement,

598    Mann-Whitney test: p>0.9999; Sum of ranks: 153.50, 122.5; Mann-Whitney U =62.50). No

599    further statistics were performed in intrinsic properties of aIC-BLA regular spiking neurons

600    representing (Figure 1 and Figure 3), because of the small sample size.

601    Changes in the intrinsic properties of neuronal ensembles have recently been suggested to

602    contribute to homeostatic mechanisms integrating both cellular and synaptic information (Wu et

603    al., 2021). In our current study, we randomly sampled from neuroanatomically defined LIV-VI

604    aIC-BLA projecting neurons, and even following spike sorting (Extended Figures 1-2), the

605    probability of recording from engram cells (10% of neurons within a region) would be extremely

606    low (Tonegawa et al., 2015). Importantly, the correlative nature does not exclude the possibility

607    that these changes are the consequence of representational drift (Driscoll et al., 2017).  We thus

608    set out to examine the hypothesis that applying linear dimension reduction method on the

609    complement of intrinsic properties recorded in BS LIV-VI aIC-BLA neurons would allow us to

610    distinguish between taste experiences that differ in terms of their perceived predictability (or the

611    associated probability for further aversive learning).

612

613    Principal component analysis of the profile of intrinsic properties in BS LIV-VI aIC-BLA

614    projecting neurons separates treatment groups in relation to the perceived predictability

615    of taste valence for saccharin

616    We assigned six of our treatment groups into highly predictive scenarios (Saccharin 5x, CTA

617    retrieval and Reinstatement), and low predictive scenarios (Saccharin 1x, Saccharin 2x,

618    Extinction). We used parallel analysis to select the components across the complement of

619    intrinsic properties in each treatment group, with the first three principal components (PC1-3)

620    explaining 30.84%, 17.88%, and 13.75% of the total variance, respectively, and 62.47% of the

621    variance, collectively (Figure 5-2). PC1 (Figure 5B, Extended Figure 5-2) was characterized by

29

622   strong negative loadings for Rheobase (-0.88304), sAHP (-0.85985) and mAHP (-0.82421),

623   while a positive correlation was identified for IR (0.694764). The direction of PC2 (Figure 5B,

624   Extended Figure 5-2) was positively correlated with fAHP (0.682681) and AP halfwidth

625   (0.614103) and was negatively correlated with Excitability at 350pA (-0.69587). PC3 (Figure 5B,

626   Extended Figure 5-2) positively correlated with SAG ratio (0.889949) and RMP (0.682681),

627   whereas a significant negative correlation with mAHP (-0.6095) was also identified. Unlike

628   aversive or appetitive taste memory retrieval (i.e., highly predictive), appetitive novelty or

629   extinction learning (i.e., low predictive), was associated with increased input resistance, faster

630   action potential generation and suppressed afterhyperpolarization on BS aIC-BLA neurons

631   (Figure 5). Importantly, PCA of the intrinsic properties of LIV-VI aIC-BLA projecting neurons

632   regardless of cell type (BS and RS together, Extended Figure 5-1), failed to segregate the two

633   groups of treatments. This cell-type specific profile of intrinsic properties could provide the

634   framework through which BS LIV-VI aIC-BLA projecting neurons are able to inspect the

635   gastrointestinal consequences associated with tastants over prolonged timescales, when these

636   consequences are not accurately predicted by sensory experience or memory retrieval alone

637   (Adaikkan and Rosenblum, 2015; Lavi et al., 2018; Kayyal et al., 2019).

638

## Discussion

640   Learning and memory are subserved by plasticity in both synapse strength and neuronal intrinsic

641   properties (Citri and Malenka, 2008; Sehgal et al., 2013). While Hebbian rules can explain asso-

642   ciative learning paradigms where seconds separate the CS and US (Krabbe et al., 2018), addi-

643   tional cellular-level mechanisms are needed to explain how learning operates in paradigms where

644   the time between CS and US extends to hours (Adaikkan and Rosenblum, 2015; Wu et al.,

645   2021). In this study, we demonstrate that following taste experiences, taste percept and prior ex-

646   perience are integrated in the intrinsic properties of the aIC in a time-dependent and cell-type

647   specific manner. We further show that regardless of the identity or prior history associated with

648   taste, the intrinsic properties of BS LIV-VI aIC-BLA projecting neurons encodes the perceived

649   confidence of taste valence attribution.

650   We focused on the aIC-BLA projection; a circuit causally involved in the acquisition and retriev-

651   al of CTA memories (Kayyal et al., 2019, 2021). (Kayyal et al., 2019, 2021). We examined the

652   hypothesis that excitability in aIC-BLA neurons can serve as a taste valence updating mechanism

653   enabling the prolonged ISI between CS and US in CTA learning (Adaikkan and Rosenblum,

654   2015), and/or contributes to anticipatory valence attribution (Barrett and Simmons, 2015). Our

655   basic proposition diverged from Hebb's famous postulate that cells with increased excitability

656   over hours can potentially wire together with cells conveying incoming modified valence infor-

657   mation (Hebb, 1961).

658   The confidence with which taste valence is encoded is the product of the subjectively perceived-

659   (a) appetitive or aversive nature of tastants and (b) novelty or familiarity associated with tastants

660   (Russell, 1980; Kahnt and Tobler, 2017). We first examined each axis separately and later in tan-

661   dem as to better simulate real-life scenarios. We measured the intrinsic properties of aIC-BLA

662   neurons 1 hour following taste experience – a previously established suitable time point (Jones et

663   al., 1999; Haley et al., 2020).

664   To dissociate novelty-related changes from those involving hydration, taste identity and familiar-

665   ity; we compared the intrinsic properties of aIC-BLA neurons following Water – a neutral and

666   familiar tastant, Saccharin – an innately appetitive tastant, in the context of novelty (1x) or famil-

667    iarity (5x), and Quinine – an innately aversive novel tastant (Figure 1). Excitability was high fol-

668    lowing novel saccharin exposure, but not in response to Quinine (Figure 1D). Indeed, concerted

669    activity at the aIC and BLA encodes the presence, identity and palatability of taste experiences

670    within the 2 seconds preceding swallowing (Katz et al., 2001; Grossman et al., 2008; Fontanini

671    et al., 2009). Palatability can be enhanced as a function of experience (Austen et al., 2016), but

672    can also be suppressed by sensory satiety and alliesthenia (Rolls et al., 1981; Yeomans, 1998;

673    Siemian et al., 2021). However, excitability on the projection was enhanced in response to novel-

674    ty and was suppressed following familiarization (Figure 1). Further inconsistent with palatability

675    encoding; changes in excitability captured 1 hour following novel saccharin exposure subsided 4

676    hours later (Figure 1), while excitability remained plastic even following longer periods of water

677    restriction, that could be considered monotonous (Figure 5). Deciphering whether and how aIC-

678    BLA neurons contribute to palatability processing would require *in vivo* recordings to capture

679    taste-evoked changes, within timescales that are beyond the scope and means of our current

680    study (Vincis and Fontanini, 2016).

681    The correlation identified between excitability and innate current taste valence, encouraged us to

682    examine the predictability of future outcomes following aversive taste memory retrieval. Bearing

683    in mind our previous findings using transcription-dependent activity markers at the aIC (Yian-

684    nakas et al., 2021), we hypothesized that aversive taste memory retrieval (CTA retrieval or Rein-

685    statement), would be associated with suppressed excitability compared to stimulus- and famili-

686    arity-matched controls (Saccharin 2x and Extinction). Indeed, excitability on aIC-BLA projecting

687    neurons following CTA retrieval was suppressed compared to Saccharin 2x (Figure 2B), while

688    Reinstatement, was also associated with decreased excitability compared to Extinction (Figure

689    3E). Hence, regardless of the complexity of taste memory retrieval, excitability in aIC-BLA neu-

690   rons was best expected by the subjective predictability of taste valence - increasing in response

691   to innately appetitive taste experiences in which the perceived possibility for avoidance learning

692   was high/taste valence predictability was low (Figure 4). Conversely, when the subjective confi-

693   dence with which taste valence was predicted was high, excitability on the projection remained

694   unchanged (Figures 1, 4).

695   Innately and learned aversive tastants were both associated with suppressed excitability on aIC-

696   BLA projecting neurons compared to appetitive controls, however these effects were mediated

697   through alternative mechanisms (Figures 2-4). Quinine increased fAHP on the projection com-

698   pared to saccharin, regardless of familiarity or perceived valence (Figures 1 and 4). Post-spike

699   after-hyperpolarization (AHP) has a key function in transducing the summed result of processed

700   synaptic input, directly impacting neuronal excitability in relation to both learning and aging (Oh

701   and Disterhoft, 2020). In pyramidal cells of the hippocampus and cortex, differences in fAHP are

702   mediated by the $Ca^{2+-}$ and voltage-dependent BK currents that promote repolarization at the be-

703   ginning APs trains (Shao et al., 1999). Interestingly, studies in the prefrontal cortex (PFC), have

704   shown that fear conditioning decreases excitability, whereas extinction training enhances excita-

705   bility and decreases medium- and slow AHP (Santini et al., 2008; Maglio et al., 2021). At the IC,

706   chronic ethanol consumption has shown to decrease excitability and to increase AHP (Luo et al.,

707   2021). Conversely, oxytocin-dependent signaling at the IC has been shown to promote social af-

708   fective behaviors, via increases in excitability and decreases in sAHP (Rogers-Carter et al.,

709   2018). Further studies would be necessary to fully address this, but our findings could indicate

710   that enhanced fAHP is induced by innately aversive tastants or quinine specifically.

711   Unlike Quinine, CTA memory retrieval, was associated with increased AP amplitude and SAG

712   ratio, as well as decreased IR in BS LIV-VI aIC-BLA projecting neurons, compared to control

33

713   animals (Figure 2, Extended Figure 2-1). On the other hand, the suppressed excitability in the

714   Reinstatement group compared to Extinction was characterized by decreased AP threshold, in-

715   creased τ, and decreased sAHP (Figures 3, 4, Extended Figure 3-1). The hyperpolarization-

716   activated, cyclic nucleotide-gated current ($I_h$) regulates membrane depolarization following hy-

717   perpolarization (Hogan and Poroli, 2008; Shah, 2014). The opening of HCN channels generates

718   an inward current, that modulates AHP, RMP and IR in cortical pyramidal and PV interneurons

719   (Yang et al., 2018). However, conductance through $I_h$ channels, regulates synaptic integration at

720   the soma of pyramidal neurons, by suppressing excitability, decreasing IR, and increasing τ (Ho-

721   gan and Poroli, 2008). Evidence indicates that this dichotomous impact of HCN channels on neu-

722   ronal excitability, is mediated by A-type K channels at the dendrites (Mishra and Narayanan,

723   2015), and M-type channels at the soma (Hu et al., 2007). Notably, AP half-width was signifi-

724   cantly increased in the Extinction and Reinstatement groups that had undergone extinction train-

725   ing, compared to all other saccharin-treated groups (Figure 4F). Mechanistically, this effect could

726   reflect changes in the distribution and/or the properties of voltage- or calcium-gated ion channels

727   (Faber and Sah, 2002; Grubb and Burrone, 2010; Kuba et al., 2010). Such broadening of spike

728   width has also been reported in infralimbic PFC neurons projecting to the amygdala in response

729   to extinction training (Senn et al., 2014). PV-dependent restriction of excitability and burst fir-

730   ing, is instrumental in experience-dependent plasticity in the amygdala (Morrison et al., 2016),

731   the hippocampus (Donato et al., 2013; Xia et al., 2017) and visual cortex (Yazaki-Sugiyama et

732   al., 2009; Kuhlman et al., 2013). Conversely, in the striatum, PV interneurons restrict bursting,

733   calcium influx, and synaptic plasticity to appropriate temporal windows that facilitate learning,

734   but not retrieval (Owen et al., 2018). Elegant recent studies report that rapid eye movement sleep

735   is associated with a PV-dependent somatodendritic decoupling in pyramidal neurons of the PFC

736  (Aime et al., 2022). At the IC, the maturation of GABAergic PV circuits is key for multisensory

737  integration and pruning of cross-modal input to coordinate the detection of relevant information

738  (Gogolla et al., 2014). Activation of IC PV disrupts the expression of taste-guided goal-directed

739  behavior (Vincis et al., 2020), and enhances taste-guided aversive responses (Yiannakas et al.,

740  2021). Our findings could be indicative of a prediction-dependent decoupling mechanism at the

741  IC, whereby the restriction of bursting activity in LIV-VI aIC-BLA neurons impinges on innate

742  drives towards the tastant and further learning, depending on prior experience.

743  We further probed our results and hypotheses using PCA and attempted to segregate behaviors

744  based on the perceived ability of the CS to predict the consequences of sensory experience, and

745  the probability for further learning (Figure 5). We focused on BS LIV-VI aIC-BLA projecting

746  neurons since bursting has been implicated in coincidence detection by deep-layer neurons

747  (Boudewijns et al., 2013; Shai et al., 2015), as well as the encoding of novelty and valence relat-

748  ing to different modalities (Song et al., 2015; Dunn et al., 2018; Yousuf et al., 2019). Our PCA of

749  intrinsic properties in BS LVI-VI aIC-BLA projecting neurons demonstrated that distinct plastici-

750  ty rules are at play depending on the balance between the probability for associative learning and

751  the certainty with which taste predicts the valence of experience during retrieval (Figure 6). We

752  propose that increased excitability and reduced fAHP on BS LIV-VI aIC-BLA projecting neu-

753  rons might represent a transient neuronal state in the absence of adequate predictive cues for the

754  outcome of taste experiences (Adaikkan and Rosenblum, 2015).

755  The IC has long been considered crucial for interoception, which is increasingly understood to be

756  supported by distinct direct or indirect functional bidirectional connectivity. Indeed, interoceptive

757  inputs relating to the processing, or anticipation of physiological states of hydration and satiety

758  manifest at the pIC (Livneh et al., 2017, 2020; Livneh and Andermann, 2021). However, this is

35

759    rarely the case when it comes to physiological hydration or satiety inputs and the aIC, that has is

760    primarily involved in interoceptive processes in the context food poisoning or CTA (Chen et al.,

761    2020; Wu et al., 2020). As other studies currently in press demonstrate, hydration correlates and

762    requires suppressed activity in aIC-BLA and increases in pIC-BLA CB1 receptor-expressing

763    neurons (Zhao et al., 2020; Nicolas et al., 2022). Under uncertain conditions that are associated

764    with greater potential significance, recruitment of the aIC is thought to contribute to attention,

765    effort, and accurate processing (Lovero et al., 2009), as to identify better response options

766    (Preuschoff et al., 2008). In agreement with earlier computational models of the cortical connec-

767    tivity (Mumford, 1991, 1992), recent work indicates that the aIC facilitates prediction-related

768    encoding driven by hedonics, rather than homeostatic needs (Darevsky et al., 2019; Price et al.,

769    2019; Darevsky and Hopf, 2020). Our results, propose a cellular framework for such an emo-

770    tional predictive function at the aIC. Future studies will further explore whether and how the in-

771    terplay between such distinct mechanisms at the aIC, enables its complex role in learning,

772    memory, and decision-making.

773

774    Conflict of interest statement

775    Authors report no conflict of interest.

776

777    Data availability

778    All data generated or analyzed during this study are included in the manuscript and supporting

779    files. Source data files have been provided for all figures.

36

780

## Author contributions

782  SKC and AY led the project. AY, SKC and KR designed the research. KR supervised the research. SKC, AY, HK, and MK performed the research. SKC, AY, LM, RS, FC, and SS analyzed the data. AY, SKC and KR drafted the paper. All authors reviewed and contributed to the manuscript.

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## Figure legends

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

A) Diagrammatic representation of experimental procedures. Following surgery and stereotaxic delivery of ssAAV_retro2-hSyn1-chi-mCherry-WPRE-SV40p(A) into the BLA, mice were allowed 4 weeks of recovery. Animals were subsequently assigned to treatment groups and trained to drink from pipettes (see Methods). We compared the intrinsic properties of LIV-VI aIC-BLA neurons among the Water (n=6 animals, 23 cells), Saccharin 1x (n=5 animals, 20 cells), Saccharin 1x(4h) (n=4 animals, 17 cells), Saccharin 5x (n=6 animals, 18 cells) and Quinine 1x groups (n=4 animals, 19 cells), as well as a Cage control group (n=4 animals, 19 cells) that underwent surgery and stereotaxic delivery of ssAAV_retro2-hSyn1-chi-mCherry-WPRE-SV40p(A) at the BLA without water restriction.

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

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

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

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1144  ^^p<0.01, ^^^ p<0.001; Saccharin 1x vs. Quinine 1x: $ p<0.05, $$p<0.01; Saccharin 1x vs.

1145  Saccharin 5x: - p<0.05; Saccharin 1x (4hr) vs. Saccharin 5x : + p<0.05.

1146  E) Representative of all fAHP measurements in response to 500 msec step current injections.

1147  Scale bars 20 mV vertical and 50 msec horizontal.

1148  F) Representative of all action potential properties were taken. Scale bars 20 mV vertical and 5

1149  msec horizontal.

1150  G) Measurements for all input resistance, sag ratio and membrane time constants were analyzed

1151  in response to 1 sec, -150pA step current injection. P- peak voltage, S- steady state voltage. Scale

1152  bars 5 mV vertical and 100 msec horizontal.

1153  H) Significant differences were observed among the treatment groups in terms of fAHP. Cage

1154  control (9.191 ± 1.449 mV), Water (8.150 ± 0.8288 mV), Saccharin 1x (3.016 ± 0.9423 mV),

1155  Quinine 1x (13.58 ± 1.562 mV) Saccharin 5x (8.158 ± 1.356 mV), Saccharin 1x (4hrs) (5.989 ±

1156  1.074 mV), One-Way ANOVA, p<0.0001.

1157  I) Action potential half-width in the Saccharin 1x group (0.6005 ± 0.03260 msec) was

1158  significantly decreased compared to Saccharin 1x (4hr) 90.7765 ± 0.03641 msec), One-Way

1159  ANOVA, p=0.0065.

1160  J) The membrane time constant was significantly different between the Saccharin 1x (14.82 ±

1161  1.485 msec) and Saccharin 1x (4hrs) (26.21 ± 2.421 msec) groups and Cage control (15.03 ±

1162  1.376 msec), One-Way ANOVA, p=0.0005.

1163  For panels 1D, H, I and J: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

1164  All data are shown as mean ± SEM.

1165

1166    Figure 2: Learned aversive taste memory retrieval suppresses the excitability of LIV-VI

1167    aIC-BLA projecting neurons

1168    A) Experimental design of behavioral procedures conducted to compare the intrinsic properties

1169    of LIV-VI aIC-BLA neurons following learned aversive taste memory retrieval (CTA retrieval -

1170    n=8 animals, 27 cells), appetitive retrieval for the same tastant (Saccharin 2x - n=5 animals, 20

1171    cells).

1172    B) Mice showed a significantly reduced saccharin consumption following learned aversive

1173    memory retrieval (N=8) compared to appetitive retrieval mice (n=5) group. p=0.0085, Mann

1174    Whitney test.

1175    C) Representative traces of LIV-VI aIC-BLA projecting neurons from the two treatment groups.

1176    Scale bars 20 mV vertical and 50ms horizontal from 300 pA step.

1177    D) The excitability of LIV-VI aIC-BLA in the Saccharin 2x group was significantly enhanced

1178    compared to CTA retrieval. Two-way repeated measures ANOVA, Current x Treatment:

1179    $p<0.0001$.

1180    E) Representative traces showing action potential measurements for both groups. Scale bar 20

1181    mV vertical and 2ms horizontal.

1182    F) Representative traces showing the input resistance and sag ratio measurements. Scale bar 10

1183    mV vertical and 100ms horizontal.

1184    G) Action potential amplitude in the CTA retrieval (56.21 ± 0.9978 mV) group was increased

1185    compared to Saccharin 2x (49.14 ± 1.568 mV), p=0.0005, Mann Whitney test.

49

1186   H) Input resistance in the CTA retrieval group (136.4 ± 9.064 MΩ) was significantly decreased

1187   compared to Saccharin 2x (181.1 ± 11.7 MΩ). p=0.0036, Unpaired t test.

1188   I) SAG ratio following CTA retrieval (13.41 ± 1.31) was significantly enhanced compared to

1189   Saccharin 2x (7.815 ± 1.176). p=0.0037, Unpaired t test.

1190   Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

1191

1192   Figure 3: Extinction of CTA enhances, whereas reinstatement suppresses the excitability

1193   of LIV-VI aIC-BLA projecting neurons

1194   A) Experimental design of behavioral procedures conducted to compare the intrinsic properties

1195   of LIV-VI aIC-BLA neurons following CTA Extinction (n=5, animals, 14 cells) and

1196   Reinstatement (n=3 animals, 15 cells).

1197   B) The graph showing the reduced aversion following the successful extinction in both treatment

1198   groups.

1199   C) Data showing the saccharin consumption on the test day following successful extinction and

1200   Reinstatement of CTA. CTA reinstated mice showed significantly reduced saccharin

1201   consumption compared to extinguished mice. p=0.0179, Mann Whitney test.

1202   D) Representative traces of LIV-VI aIC-BLA projection neurons firing from the two treatment

1203   groups. Scale bars 20 mV and 50ms horizontal from 300 pA step.

1204   E) Excitability in LIV-VI aIC-BLA neurons was significantly different among the treatment

1205   groups. Two-Way repeated measures ANOVA, Current x Treatment: p<0.0001.

1206   F) Action potential threshold in the Reinstatement group (-29.43 ± 1.731 mV) was enhanced

50

1207    compared to Extinction (-36.06 ± 1.481 mV). p=0.0076, Unpaired t test.

1208    G) The membrane time constant following Reinstatement (25.48 ± 1.58 msec) was significantly

1209    enhanced compared to Extinction (17.55 ± 2.684 msec, p=0.047). p=0.0153, Unpaired t test.

1210    For panels 5D-F: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

1211    All data are shown as mean ± SEM.

1212

1213    Figure 4: Innately aversive taste is correlated with high fAHP, and prolonged conflicting

1214    experiences is correlated with an increased AP half-width in LIV-VI aIC-BLA projecting

1215    neurons

1216    We compared the intrinsic properties of LIV-VI aIC-BLA neurons among the Saccharin 1x (n=5

1217    animals, 20 cells), Quinine 1x (n=4 animals, 19 cells), Saccharin 2x (n=5 animals, 20 cells), CTA

1218    retrieval (n=8, 27 cells), Extinction (n=5 animals, 14 cells) and Reinstatement (n=3 animals, 15

1219    cells) groups.

1220    A) Groups associated with positive taste valence (Saccharin 1x, Saccharin 2x, Extinction),

1221    exhibited significantly increased excitability compared to innate or learned negative taste valence

1222    groups (Quinine 1x, CTA retrieval and Reinstatement). Two-way repeated measures ANOVA,

1223    Current x Treatment: p<0.0001; Saccharin 2x vs. CTA retrieval: *p; Saccharin 2x vs.

1224    Reinstatement: #p: Saccharin 2x vs Quinine 1x: p$; Saccharin 1x vs. CTA retrieval: p^;

1225    Saccharin 1x vs. Quinine 1x: p%; Saccharin 1x vs reinstatement: p+; Extinction vs. CTA

1226    retrieval: p@; Extinction vs. Reinstatement: p&; Extinction vs. Quinine 1x: p-.

1227    B) fAHP was significantly enhanced in response to Quinine 1x (13.56± 1.562 mV) compared to

51

1228   all other groups. Significant differences were also observed between Saccharin 1x (3.016 ±

1229   0.9423 mV), Saccharin 2x (5.223 ± 0.8217 mV), and CTA retrieval (7.97 ± 1.018 mV,

1230   p=0.0036). Extinction (4.731 ± 1.021 mV) and Reinstatement (5.932 ± 1.292 mV). One-Way

1231   ANOVA, p<0.0001.

1232   C) Input resistance was significantly different between Saccharin 2x (181.1 ± 11.7 MΩ) and CTA

1233   retrieval (136.4 ± 9.064 MΩ), p= 0.0352. Conversely, Input resistance in Saccharin 1X (145.8 ±

1234   12.56), Quinine 1X (146 ± 9.094), Extinction (151.1 ± 15.63), and Reinstatement groups was

1235   similar. One-Way ANOVA, p= 0.0213.

1236   D) SAG ratio was significantly different between Saccharin 2x (7.815 ± 1.176) and CTA retrieval

1237   (13.41 ± 1.31), p= 0.0209. Conversely, SAG ratio in Saccharin 1x (10.89 ± 1.621), Quinine 1x

1238   (12.13 ± 1.23), Extinction (12.37 ± 1.471) and Reinstatement (9.245 ± 0.884) groups was similar

1239   One-Way ANOVA, p= 0.0286.

1240   E) Action potential amplitude in the Quinine 1x group (57.11 ± 1.376 mV), and CTA retrieval

1241   (56.21 ± 0.9978 mV), was significantly increased compared to Saccharin 2x (49.14 ± 1.568 mV,

1242   p= 0.0175, and 0.0229, respectively). Conversely, action potential attitude in the Saccharin 1x

1243   (52.03 ± 1.308 mV), Extinction (55.09 ± 2.122 mV) and Reinstatement (53.1 ± 2.906 mV)

1244   groups was similar. One-Way ANOVA, p = 0.0061.

1245   F) Action potential half-width following Extinction (0.7386 ± 0.03145 msec) and Reinstatement

1246   (0.8187 ± 0.06929 msec) was elevated compared to Saccharin 1x (0.6005 ± 0.03260 msec),

1247   Saccharin 2x (0.5780 ± 0.02994 msec) as well as CTA retrieval (0.5959 ± 0.02080 msec, but no

1248   with Quinine 1x (0.6300 ± 0.03555 msec). One-Way ANOVA, p = 0.0002.

1249   G) The membrane time constant in the Saccharin 1x (14.82 ± 1.485 msec) group was

1250 significantly suppressed compared to Reinstatement (25.48 ± 1.58 msec, p= 0.0043) groups was.

1251 Differences between CTA retrieval (20.96 ± 1.724 msec, p=0.0189), Quinine 1x (21.55 ± 1.638

1252 msec), Saccharin 2x (19.28 ± 1.837 msec) and Extinction (17.55 ± 2.684 msec) groups failed to

1253 reach significance. One-Way ANOVA, p = 0.0047.

1254

1255 Figure 5: The intrinsic properties of burst-spiking LIV-VI aIC-BLA projecting neurons

1256 represent taste experience and the probability for further learning

1257 A) Data across all intrinsic properties from BS LIV-VI aIC-BLA neurons of the Saccharin 1x,

1258 Saccharin 2x and Extinction groups were combined and assigned to the Low predictive

1259 following memory group (32 BS cells). Conversely, the intrinsic properties of BS LIV-VI aIC-

1260 BLA neurons from animals having undergone CTA retrieval, 5x Saccharin, and Reinstatement

1261 were combined and assigned to the High predictive following memory group (31 BS cells). The

1262 resultant three-dimensional scatter representation of the two groups encompassed Excitability at

1263 350pA; AP amplitude, AP halfwidth, AP threshold; fAHP, mAHP, sAHP; IR, Rheobase, RMP,

1264 SAG ratio and τ in BS LIV-VI aIC-BLA neurons.

1265 B) Three-dimensional representation of the contribution of individual parameters (loadings

1266 matrix) to the principal components segregating the two groups of treatments (scores matrix).

1267

1268 Figure 6: A model for excitability changes in IV-VI aIC-BLA neurons when the taste

1269 salience is low and highly predictive following the taste memory

1270 • The unpredictability of future valence outcomes: Novel appetitive taste experiences

1271 /extinction of previously learned aversive taste experiences, increases the excitability

53

1272      (blue arrow) of aIC-BLA projecting neurons (red). This increased excitability is specific

1273      to the deep-layer aIC-BLA projecting neurons (LIV-VI).

1274    • When the taste valence is highly predictable (familiar/aversive), excitability is reduced in

1275      LIV-VI aIC-BLA projecting neurons (red arrow).

1276    • LIV-VI aIC-BLA neurons remain excitable facilitating the association of the taste

1277      memory trace with visceral pain when the stimulus is not adequately predictive of the

1278      outcome of the sensory experience.

1279

## 1280 Extended figures and Tables

1281

1282 Extended Figure 1-1: Histological verification of rAAV-mCherry virus expression and

1283 locations of whole- cell patch clamp recordings

1284 A) A representative image showing the distribution of retrograde injections into the BLA and

1285 aIC-BLA projection neuron at aIC.

1286 B) Mean localization of BLA projecting neurons of the agranular aIC used for

1287 electrophysiological whole cell recordings.

1288 Extended Figure 1-2: The ratio of burst-spiking and regular spiking LIV-VI aIC-BLA

1289 projecting neurons changes in relation to the uncertainty associated with taste experiences

1290 A) Representative traces from Burst (BS) and Regular (RS) spiking LIV-VI aIC-BLA projecting

1291 neurons in response to rheobase current injections. The neurons showing doublets or triplets in

1292   response to rheobase current injection were considered BS. The neurons showing single spike in

1293   response to rheobase current injection considered RS. Scale bars 20 mV and 100 msec.

1294   B) Pie charts showing the change in the ratio of BS vs RS LIV-VI aIC-BLA projection neurons,

1295   expressed as a percentage of the sampled population across the Saccharin 1x, Saccharin 2x,

1296   Saccharin 5x, CTA retrieval, Extinction, and Reinstatement groups.

1297   C) Heat map summary of the change in the ratio of BS vs RS LIV-VI aIC-BLA projection

1298   neurons, expressed as a percentage of the sampled population across the six treatment groups.

1299

1300   Extended Figure 1-3: Appetitive novel taste alters the intrinsic properties of burst spiking

1301   LIV-VI aIC-BLA neurons

1302   We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons among the Cage

1303   control (n=13 cells), Water (n= 11cells), Saccharin 1X (n=17 cells), Quinine 1x (n=9 cells),

1304   Saccharin 5x (n=10 cells) and Saccharin 1x (4hrs, n=6 cells).

1305   A) Excitability in BS LIV-VI aIC-BLA was not significantly different among the treatment

1306   groups. Two-Way repeated measures ANOVA, Current x Treatment: $p<0.0001$, Group interaction

1307   $p= 0.0666$.

1308   B) fAHP was significantly enhanced in Quinine 1x (13.67 ±2.681 mV) and Saccharin 5x (11.30

1309   ±1.727 mV) BS neurons compared to Saccharin 1x BS neurons (2.870 ±1.044 mV). One-Way

1310   ANOVA, $P= 0.0004$.

1311   C) Action potential amplitude was significantly different between the groups. Cage controls

1312   (56.27 ±1.147 mV), Water (54.21 ±1.572 mV), Saccharin 1x (51.64 ± 1.473 mV), Quinine 1X

1313   (58.86 ± 2.003 mV), Saccharin 5x (58.40 ± 1.812 mV), and Saccharin 1x (4hr) (46.79 ± 4.359

55

1314    mV). One-Way ANOVA, P= 0.0097.

1315    D) Action potential half-width in BS LIV-VI aIC-BLA neurons of the Saccharin 1x (4 hrs.)

1316    group (0.8850 ± 0.05943ms) was increased compared to the Saccharin 1x (1hr) group - 0.5976 ±

1317    0.03555ms. One-Way ANOVA, P=0.0139.

1318    E) Action potential threshold was not significantly different between the groups. Cage control (-

1319    31.83± 2.971 mV), Water (-29.27 ± 2.060 mV), Saccharin 1x (-30.73 ± 2.385 mV), Quinine 1x (-

1320    29.35 ± 3.071 mV), Saccharin 5x (-30.38 ± 2.493 mV), and Saccharin 1x (4hr) (-34.61 ± 2.174

1321    mV). One-Way ANOVA, P= 0.7652.

1322    F) Input resistance was similar among the different treatment groups. Cage control (118.4 ±

1323    9.771 MΩ), Water (136.5 ± 14.40 MΩ), Saccharin 1x (146.6 ±14.22 MΩ), Quinine 1x (139.2 ±

1324    16.86 MΩ), Saccharin 5x (156.1 ± 22.85 MΩ), and Saccharin 1x (4hr) (154.9 ± 22.41 MΩ).

1325    One-Way ANOVA, P= 0.6304.

1326    G) SAG ratio was not significantly different between the groups. Cage control (14.91 ± 2.195),

1327    Water (8.751± 2.021), Saccharin 1x (11.67±1.790), Quinine 1x (14.15 ± 2.159), Saccharin 5x

1328    (11.92 ± 3.395), and Saccharin 1x (4hr) (14.99 ± 2.770). One-Way ANOVA, P= 0.2232.

1329    H) Membrane time constant was significantly different among the treatment groups. Cage

1330    control (14.71±1.944 msec), Water (18.03 ± 2.309 msec), Saccharin 1x (14.27 ± 1.666 msec),

1331    Quinine 1x (23.21 ± 2.717 msec), Saccharin 5x (17.11 ± 2.296 msec), and Saccharin 1x (4hr)

1332    (26.09 ± 5.331msec). One-Way ANOVA, P= 0.0321.

1333    Data are shown as mean ± SEM. *p<0.05, **p<0.01.

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1337 Extended Figure 2-1: Learned aversive taste memory retrieval suppresses the excitability

1338 of burst spiking LIV-VI aIC-BLA neurons

1339

1340 We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons following

1341 Saccharin 2xs (BS=13, RS=7, cells) and CTA memory retrieval (BS=12, RS=15, cells).

1342 A) Excitability in BS LIV-VI aIC-BLA neurons was significantly reduced in the CTA retrieval

1343 group compared to Saccharin 2x. Two-Way repeated measures ANOVA, Current x Treatment:

1344 p<0.0001.

1345 B) Input resistance in BS LIV-VI aIC-BLA neurons was significantly enhanced in the Saccharin

1346 2x (180.3 ± 15.15 MΩ) compared to CTA retrieval (110.9 ± 12.98 MΩ). Unpaired t test, p=

1347 0.0022.

1348 C) Action potential amplitude in BS LIV-VI aIC-BLA neurons was significantly increased in the

1349 CTA retrieval group compared to Saccharin 2x (46.18 ± 1.666 mV), and CTA retrieval (57.87 ±

1350 1.678 mV). Mann Whitney test, p<0.0001.

1351 D) SAG ratio in BS LIV-VI aIC-BLA neurons was significantly suppressed in the Saccharin 2x

1352 (7.017 ± 1.317) compared to CTA retrieval (16.8 ± 1.869). Mann Whitney test, p= 0.0005.

1353 E) Representative traces of RS LIV-VI aIC-BLA neurons firing from the two treatments. Scale

1354 bars 20 mV vertical and 50ms horizontal in response to 150 pA step current.

1355 F) Excitability in RS LIV-VI aIC-BLA neurons was similar in the CTA retrieval and Saccharin

57

1356  2x. Two-Way repeated measures ANOVA, Current x Treatment: p= 0.0953.

1357  G) Input resistance in RS LIV-VI aIC-BLA neurons was not significantly different in between

1358  the groups. Saccharin 2x (182.6 ± 19.62 MΩ), and CTA retrieval (156.7 ± 10.11 MΩ). Mann

1359  Whitney test, p >0.9999.

1360  H) SAG ratio in RS LIV-VI aIC-BLA neurons was not significantly different between the

1361  groups. Saccharin 2x (9.297 ± 2.347), and CTA retrieval (10.71 ± 1.536). Mann Whitney test, p=

1362  0.5815.

1363  I) Action potential amplitude in RS LIV-VI aIC-BLA neurons was not significantly different

1364  between the groups. Saccharin 2x (54.62 ± 2.058 mV), and CTA retrieval (54.89 ± 1.13 mV).

1365  Mann Whitney test, p>0.9999.

1366  J) AP half-width in RS LIV-VI aIC-BLA neurons was significantly reduced following CTA

1367  memory retrieval (0.5633 ± 0.01703msec) compared to the Saccharin 2x (0.6614 ±

1368  0.04149msec). Mann Whitney test, p= 0.0200.

1369  K) Membrane time constant was similar in both treatment groups. Saccharin 2x RS (18.36 ±

1370  2.842ms), and CTA memory retrieval RS (24.08 ± 2.023msec). Mann Whitney test, p= 0.0777.

1371  Data are shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

1372

1373  Extended Figure 3-1: Extinction of CTA enhances, excitability of burst spiking LIV-VI

1374  aIC-BLA projecting neurons

1375

1376  We compared the intrinsic properties of BS and RS LIV-VI aIC-BLA neurons following the

58

1377    Extinction (BS=11, RS=3, cells) and Reinstatement (BS=10, RS=5, cells).

1378    A) Excitability in BS LIV-VI aIC-BLA was significantly enhanced in Extinction group

1379    comparing to Reinstatement. Two-Way repeated measures ANOVA, Current x Treatment:

1380    $p<0.0001$.

1381    B) sAHP in BS LIV-VI aIC-BLA neurons was significantly enhanced in the Extinction group (-

1382    $2.104 \pm 0.4466$ mV) compared to Reinstatement (-3.804 $\pm$ 1.339 mV) neurons. Mann Whitney

1383    test, $p=0.0230$.

1384    C) Action potential threshold in BS LIV-VI aIC-BLA neurons was significantly reduced in the

1385    Extinction group (-37.41 $\pm$ 1.636 mV) compared to Reinstatement (-27.5 $\pm$ 2.195 mV). Unpaired

1386    t test, $p= 0.0016$.

1387    D) Input resistance in BS LIV-VI aIC-BLA neurons was similar in the two treatment groups.

1388    Extinction (131.1 $\pm$ 13.93 M$\Omega$) and Reinstatement BS (157.4 $\pm$ 10.56 M$\Omega$). Mann Whitney test,

1389    $p= 0.1321$.

1390    E) SAG ratio in BS LIV-VI aIC-BLA neurons was enhanced following Extinction (13.69 $\pm$

1391    1.541) neurons compared to Reinstatement BS (9.124 $\pm$ 1.03). Unpaired t test, $p= 0.0262$.

1392    F) Membrane time constant in BS LIV-VI aIC-BLA neurons was significantly reduced in the

1393    Extinction group (14.52 $\pm$ 2.714 msec) compared to Reinstatement (26.93 $\pm$ 1.893) neurons.

1394    Mann Whitney test, $p= 0.0062$.

1395    G) Representative traces of RS LIV-VI aIC-BLA firing from two treatment groups. Scale bars 20

1396    mV vertical and 50 msec horizontal in response to 150 pA current step.

1397    H) Excitability of RS LIV-VI aIC-BLA neurons in both treatment groups.

1398    I) Input resistance in RS LIV-VI aIC-BLA neurons was similar in the Extinction (224.2 ± 21.29

1399    MΩ) and Reinstatement (221.2 ± 18.9 MΩ) groups.

1400    J) SAG ratio in RS LIV-VI aIC-BLA neurons was not different between the Extinction (7.515 ±

1401    2.666) and Reinstatement (9.486 ± 1.846) groups.

1402    K) Membrane time constant in RS LIV-VI aIC-BLA neurons was not different between the

1403    Extinction (28.69 ± 2.138msec) and Reinstatement groups (22.58 ± 2.632msec).

1404

1405    Extended Figure 5-1: PCA showing Burst vs Regular spiking LIV-VI aIC-BLA neurons

1406    all range of excitability vs 350 pA only

1407    A) PCA of BS and RS LIV-VI aIC-BLA neurons all range of excitability (50-350 pA and all

1408    other intrinsic properties measured). Sampled population across six treatment groups (Saccharin

1409    1x, Saccharin 2x, Saccharin 5x, CTA retrieval, Extinction, Reinstatement).

1410    B) PCA of BS and RS LIV-VI aIC-BLA neurons excitability of 350 pA only and all other

1411    intrinsic properties measured. Sampled population across six treatment groups (Saccharin 1x,

1412    Saccharin 2x, Saccharin 5x, CTA retrieval, Extinction, Reinstatement).

1413

1414    Extended Figure 5-2: PCA variable contributions and component loadings of BS and RS

1415    LIV-VI aIC-BLA projecting neurons

1416    A) Column chart demonstrating the individual and cumulative proportion of the variance

1417    accounted by principal components following PCA of BS LIV-VI aIC-BLA projecting neurons

1418    in the two groups of treatments (Saccharin 1x, Saccharin 2x, Extinction vs. CTA retrieval, 5x

1419    Saccharin, Reinstatement).

1420    B) Table summarizing the contribution of individual variables (loadings) to the coordinate value

1421    of the principal components segregating the two groups (score).

1422    C) Communalities table, demonstrating the amount of variance in each variable that is accounted

1423    for by the extraction of principal components. Initial communalities are estimates of the variance
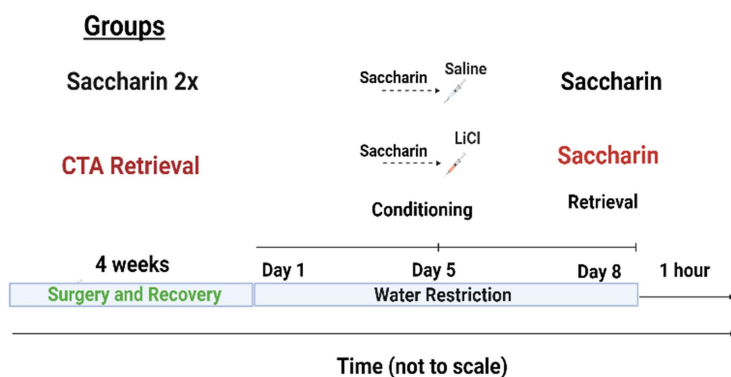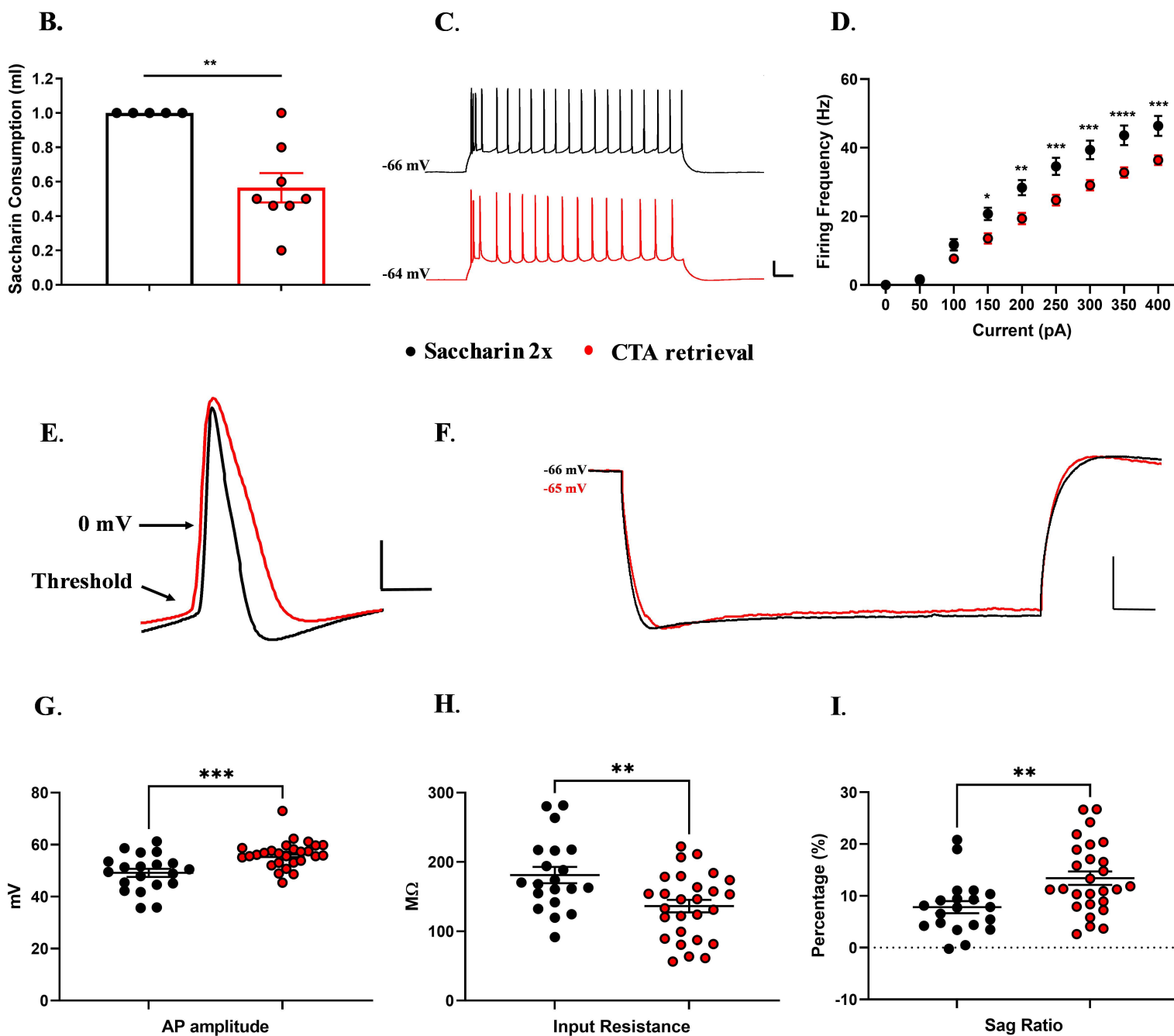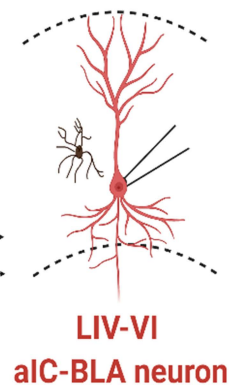
1424    in each variable accounted for by all components or factors (=1.00).

1425
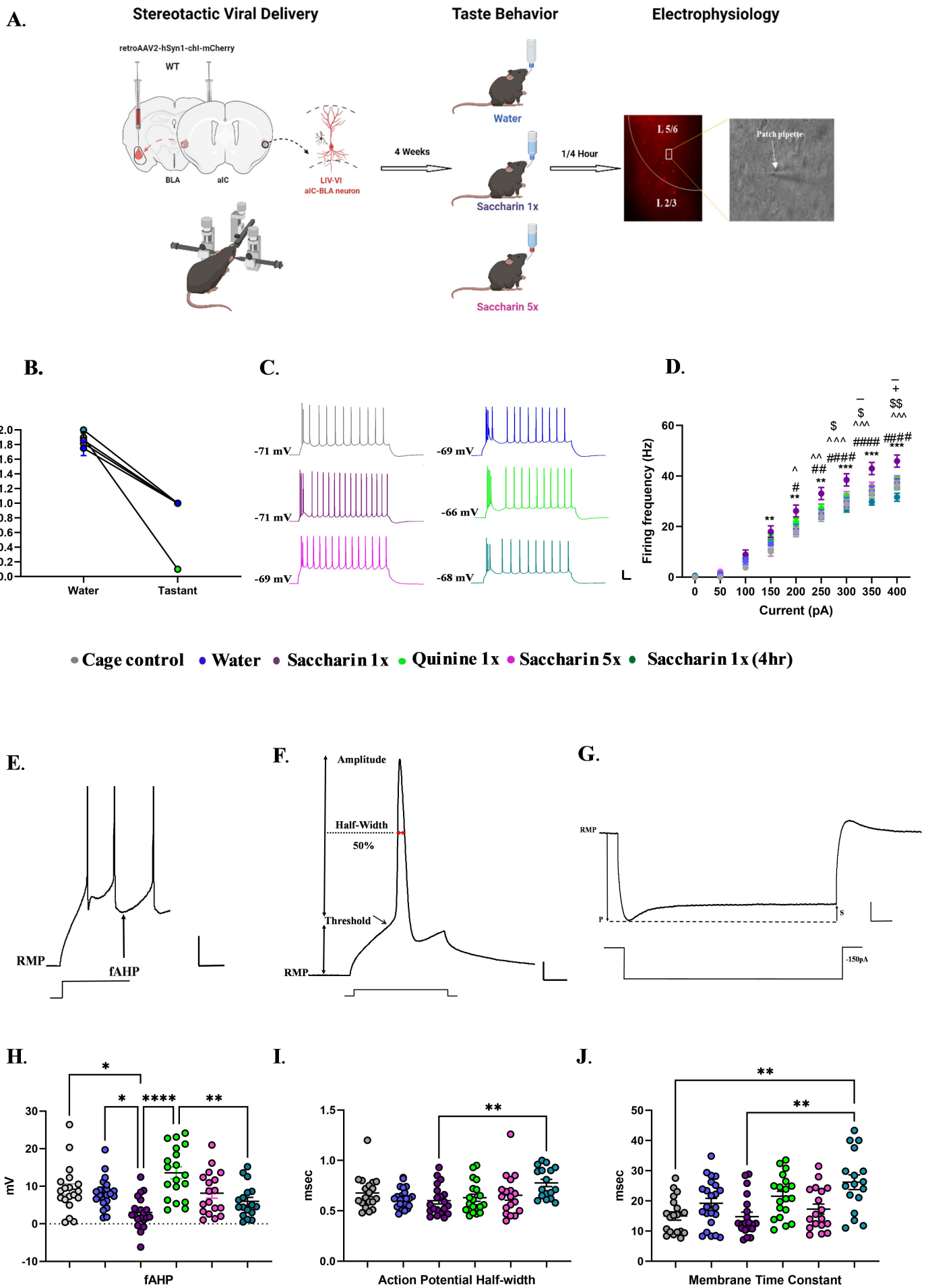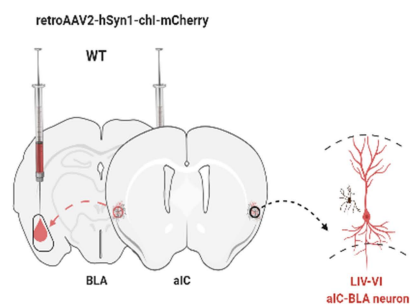
# A.

## Taste Behavioral Paradigms

### Groups

**Extinction**  Saccharin → LiCl   Saline   **Saccharin**

**Reinstatement**  Saccharin → LiCl   LiCl   **Saccharin**

Conditioning   Extinction Learning   Retrieval

4 weeks   Day 5   Days 8-16   Day 19   Day 20   1 hour

**Surgery and Recovery**   **Water Restriction**

Time (not to scale)

LIV-VI aIC-BLA neuron



# B.



Aversion Index (%) vs Extinction Trial (Days)

# C.



Saccharin Consumption (ml)

*

# D.



-64 mV

-72 mV

● **Extinction**   ● **Reinstatement**

# E.



Firing Frequency (Hz) vs Current (pA)

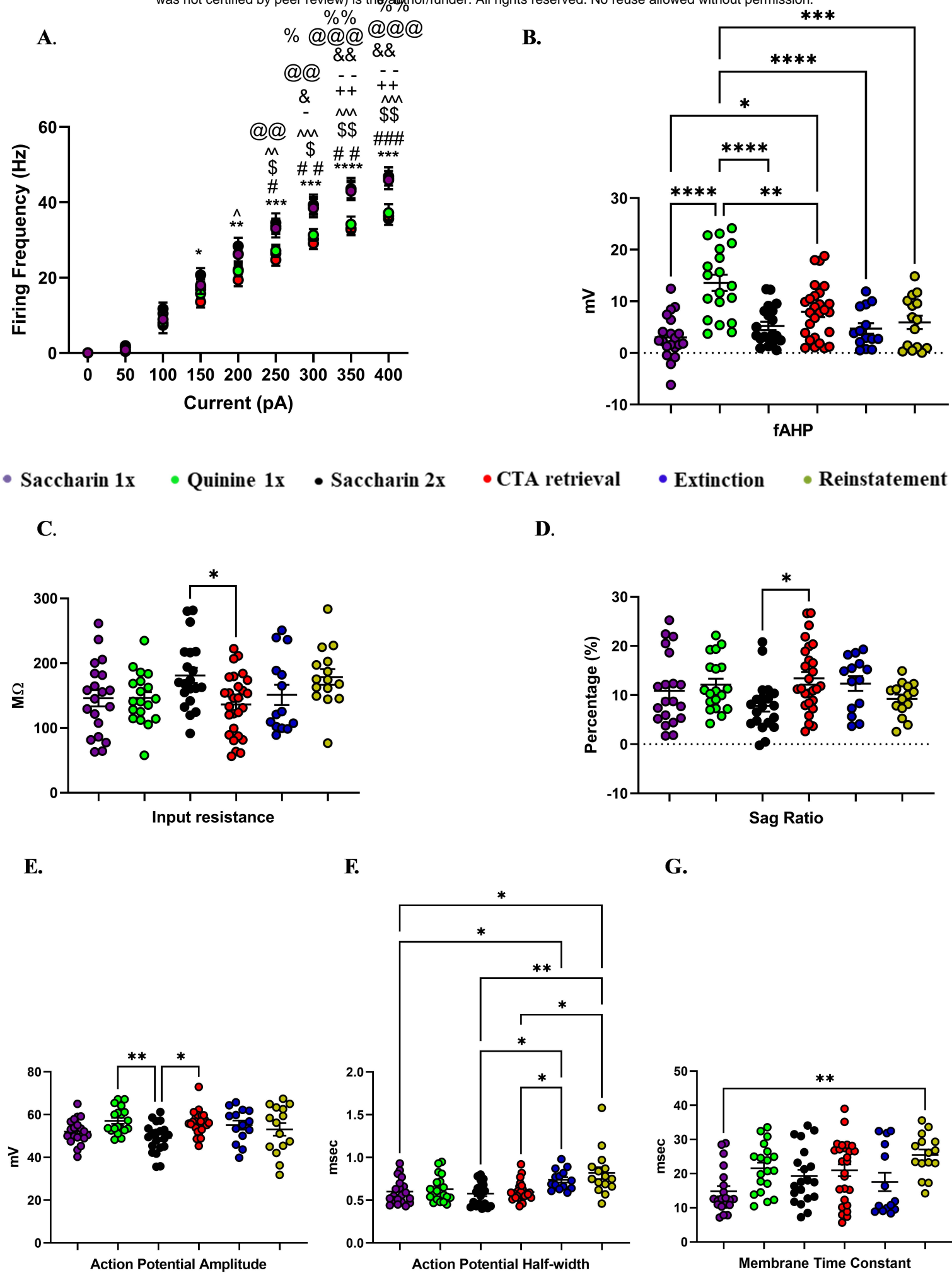* ** *** ****

# F.



mV

AP Threshold

**

# G.



msec

Membrane Time Constant

*

**A.**

Low Predictive Following memory
High Predictive Following memory

PC 3 (13.75 %)

PC 1 (30.84 %)

PC 2 (17.88 %)

**B.**

PC 3 (13.75 %)

PC 1 (30.84 %)

PC 2 (17.88 %)

RMP(mV)

Sag Ratio

350pA

AP amplitude (mV)

IR(MΩ)

AP threshold(mV)

AP Halfwidth(ms)

fAHP(mV)

Time constants(ms)

sAHP(mV)

Rheobase(pA)

mAHP (mV)

Appetitive Novel

↑ Excitability

L IV-VI aIC-BLA
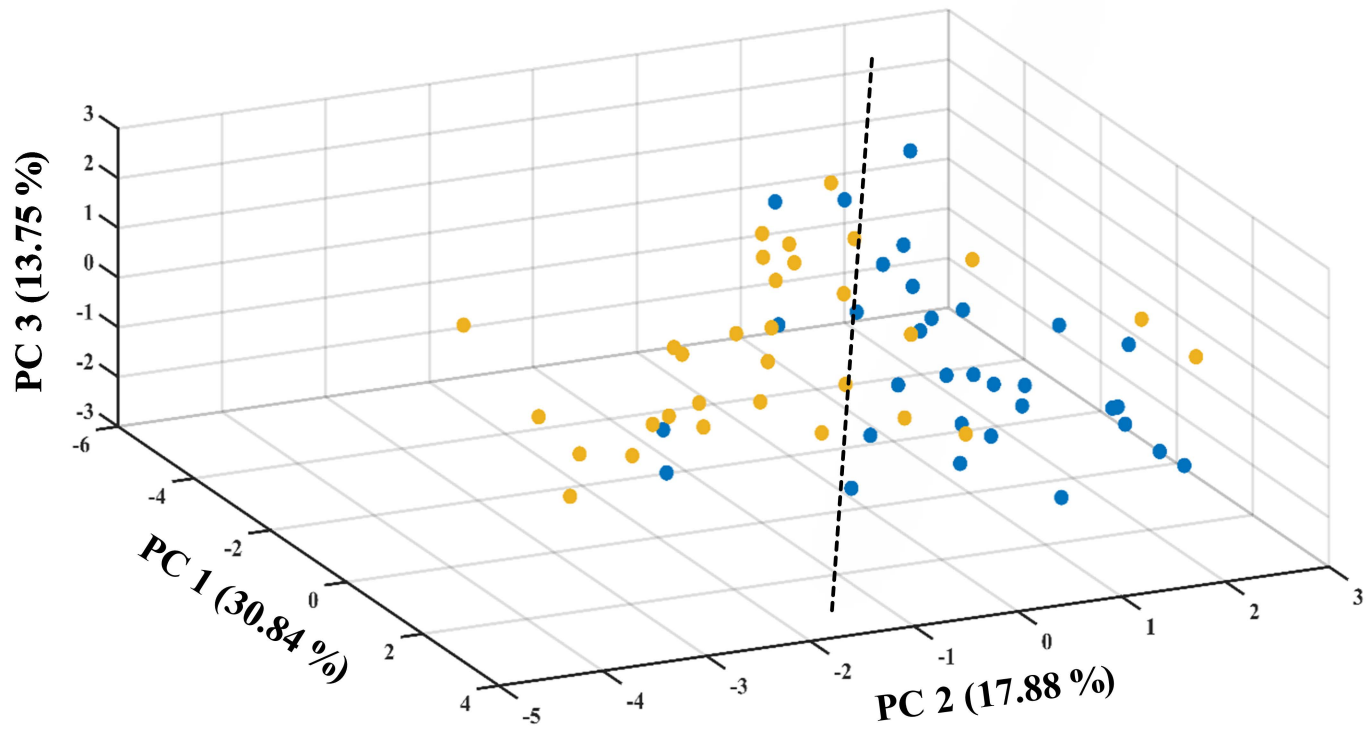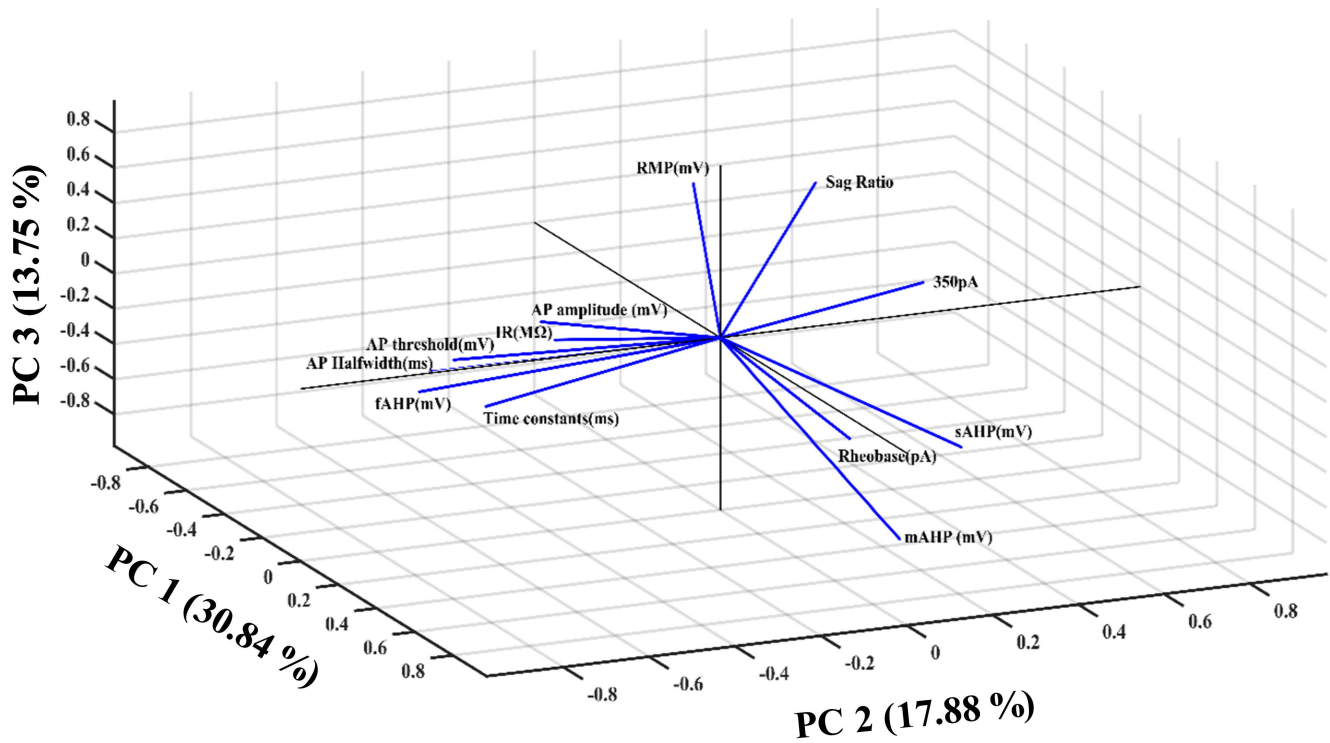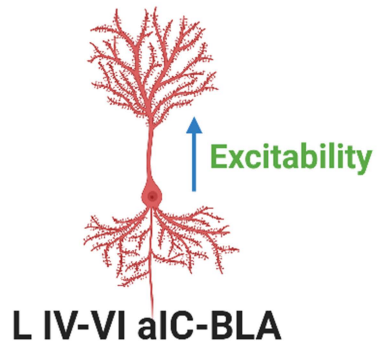
Probabilty of Learning to Avoid

High

Low

Appetitive Familiar/Aversive

↓ Excitability

L IV-VI aIC-BLA

L I

L II-III

L IV-VI

aIC

BLA