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2     **Deletion of Stk11 and Fos in mouse BLA projection neurons alters intrinsic**  
3     **excitability and impairs formation of long-term aversive memory**  
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6                   Abbreviated Title: The role of Stk11 in memory formation  
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27 **Abstract**

28 Conditioned taste aversion (CTA) is a form of one-trial learning dependent on basolateral  
29 amygdala projection neurons (BLApn). Its underlying cellular and molecular mechanisms  
30 are poorly understood, however. We used RNAseq from BLApn to identify learning-  
31 related changes in Stk11, a kinase with well-studied roles in growth, metabolism and  
32 development, but not previously implicated in learning. Deletion of Stk11 restricted to  
33 BLApn completely blocks memory when occurring prior to training, but not following it,  
34 despite altering neither BLApn-dependent encoding of taste palatability in gustatory  
35 cortex, nor transcriptional activation of BLApn during training. Deletion of Stk11 in BLApn  
36 also increases their intrinsic excitability. Conversely, BLApn activated by CTA to express  
37 the immediate early gene Fos had reduced excitability. BLApn knockout of Fos also  
38 increased excitability and impaired learning. These data suggest that Stk11 and Fos  
39 expression play key roles in CTA long-term memory formation, perhaps by modulating  
40 the intrinsic excitability of BLApn.

41

42 **Introduction**

43 Conditioned Taste Aversion (CTA) is a form of long-lasting aversive memory induced by  
44 a single pairing of exposure to an initially palatable taste with gastric malaise (Bures et  
45 al., 1998). Although multiple brain regions, including the brainstem, amygdala and the  
46 cortex, participate in various aspects of taste behavior (reviewed in Carleton et al., 2010),  
47 prior work suggests that the basolateral amygdala (BLA) plays a critical role in CTA  
48 memory. Disrupting neuronal activity within the BLA blocks the formation and retrieval of  
49 CTA memory (Yasoshima et al., 2000; Ferreira et al., 2005; Garcia-Delatorre et al., 2014;  
50 Molero-Chamizo, et al., 2017). This may reflect the fact that BLA projection neurons  
51 (BLApn) provide the principal output pathway from the amygdala to forebrain structures  
52 including the gustatory cortex and the central amygdala (Duvarci and Pare, 2014)  
53 enabling it to distribute taste valance information to these regions (Piette et al., 2012;  
54 Samuelsen et al., 2012). Consistent with this view, BLA neurons change their activity and  
55 their functional connectivity with their down-stream targets during CTA learning  
56 (Grossman et al., 2008). However, whether the BLA is a site of cellular and molecular  
57 plasticity during CTA learning, as opposed to merely gating plasticity in other structures,  
58 is not known.

59

60 Stages of memory formation are typically distinguished on the basis of duration and  
61 molecular mechanism. Short-term memory, lasting minutes to hours, requires only post-  
62 translational modification of preexisting proteins, whereas long-term memory, lasting  
63 days or longer, requires gene transcription and RNA translation, typically occurring in the  
64 hours following memory acquisition (Matthies, 1989; Alberini, 2009; Gal-Ben-Ari et al.,  
65 2012; Kandel, 2001). Production of new proteins is required to produce lasting changes  
66 in the efficacy of synaptic connections and in the intrinsic excitability of neurons, which  
67 are thought to be the cellular correlates of memory (Zhang and Linden, 2003;  
68 Mozzachiodi and Byrne, 2010; Takeuchi et al., 2014). The cellular correlates of CTA

69 learning are less completely understood than those of some other forms of learning, but  
70 the involvement of both synaptic plasticity (Li et al., 2016) and intrinsic plasticity  
71 (Yasoshima and Yamamoto, 1998; Zhou et al., 2009) have been demonstrated. CTA is  
72 known to require protein synthesis in the BLA (Josselyn et al., 2004) and to increase the  
73 expression of the activity dependent transcription factor Fos (Uematsu et al., 2015). In  
74 other behavioral paradigms, neurons increasing Fos protein undergo changes in synaptic  
75 strength and intrinsic excitability (Yassin et al., 2010; Ryan et al., 2015; Pignatelli et al.,  
76 2019) and are thought to be essential parts of the neuronal network underlying long-term  
77 memory (Tonegawa et al., 2015). However, the role of neurons expressing Fos in the  
78 BLA during CTA is unclear. Also unknown is whether CTA learning requires new  
79 transcription, and if so, the identities of the required transcripts and cellular processes  
80 they promote are not known.

81 In this study we found that new transcription in the BLA is required for CTA learning. Using  
82 RNA-seq from sorted neurons, we found that expression of the kinase Stk11, also known  
83 as LKB1, is altered following learning in BLA projection neurons (BLApn), but not in  
84 excitatory or inhibitory neurons within the GC. Stk11 is known to act as a master regulator  
85 of growth, metabolism, survival and polarity by phosphorylating 13 down-stream  
86 members of the AMP-related kinase family (Lizcano et al., 2004; Shackelford and Shaw,  
87 2009). Recent work also suggests roles for Stk11 in the nervous system, where it controls  
88 axonal specification and dynamics during development (Barnes et al., 2007; Shelly et al.,  
89 2007) and synaptic remodeling during old age (Samuel et al., 2014). Stk11 can also  
90 regulate synaptic transmission in forebrain neurons (Kwon et al., 2016), but it is not known  
91 to play a role in learning or in the regulation of intrinsic neuronal excitability.

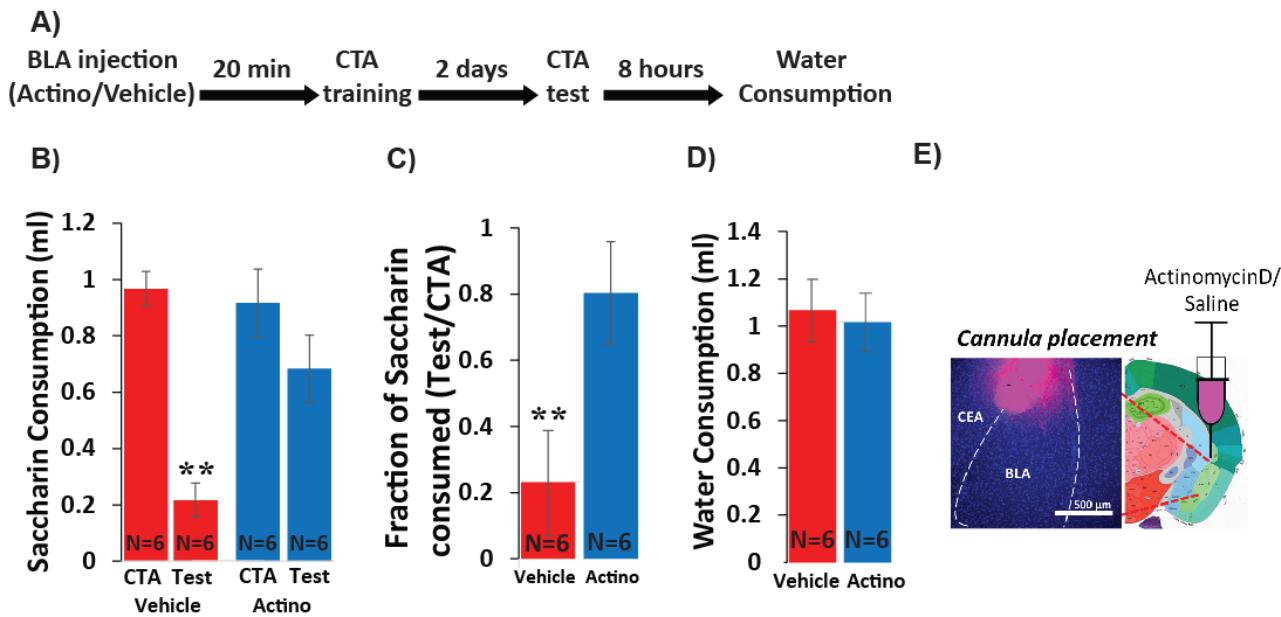
92 We find that Stk11 is required for CTA since conditional knockout from BLApn prior to  
93 training completely blocks learning. However, the same deletion performed two days after  
94 training—i.e., at a time when long-term memories have already been formed and  
95 stabilized—has no effect on subsequent memory retrieval. Deletion of Stk11 also  
96 increased the excitability of BLApn, but did not alter the ability of the BLA-GC circuit to  
97 become transcriptionally activated by training or to encode the palatability of gustatory  
98 stimuli. CTA training is associated with an opposing decrease in intrinsic excitability  
99 change in a sub-population of BLA projection neurons expressing the activity dependent  
100 gene Fos following learning. BLApn knockout of Fos also increased excitability and  
101 impaired learning. Together, these data suggest that Stk11 and Fos expression play key  
102 roles in CTA long-term memory formation, perhaps by modulating the intrinsic excitability  
103 of BLApn.

104  
105 **Results**

106 **CTA long-term memory requires BLA transcription**

107 In order to determine whether CTA requires new RNA transcription within the BLA, we  
108 inhibited transcription by injecting Actinomycin-D (1  $\mu$ l, 50 ng, bilaterally, Figure 1), a

109 widely used RNA polymerase 2 inhibitor (Alberini, 2009), into the BLA 20 min prior to CTA  
110 training, and tested memory 48 hours later. As a control, a separate group of mice  
111 received vehicle injection (1  $\mu$ l of PBS, bilaterally). CTA training consisted of 30 min of  
112 access to 0.5% saccharin followed by an intraperitoneal injection of 0.15M LiCl, 2% body  
113 weight; (Figure 1-figure supplement 1). A two-way ANOVA comparing vehicle and



114 actinomycin-D injected mice before and after training revealed significant training and

115 **Figure 1. Inhibiting BLA transcription impairs CTA Learning.** (A) Protocol for injection (1  $\mu$ l  
116 per hemisphere) of Actinomycin-D (50 ng) or vehicle (PBS with 0.02% DMSO). (B) Actinomycin  
117 D injection prior to CTA training impairs learning, expressed as a reduction in saccharin  
118 consumption between CTA and test sessions. Group and treatment effects were significant by  
119 two-way ANOVA (groups:  $F(3,20)=13.05$ ;  $p=6E-5$ ; treatment:  $F(1,20)=4.83$ ;  $p=0.04$ ; with a  
120 significant interaction:  $F(1,20)=7.4$ ;  $p=0.013$ ). Post hoc analysis (Bonferroni corrected) revealed  
121 significant reductions ( $p=1E-4$ ) of saccharin consumption (training vs. test for vehicle treated, but  
122 not for actinomycin-D treated mice ( $p=0.583$ ). (C) Fraction of saccharin consumed (Test/CTA)  
123 was significantly higher ( $t(10)=-3.36$ ;  $p=0.007$ ) following Actinomycin D treatment than vehicle,  
124 consistent with weaker memory. (D) Treatments did not differ in water consumption measured 8  
125 hours later ( $t(10)=0.279$ ;  $p=0.794$ ) suggesting this does not account for differences in  
126 consumption during the test. \*\* $p<0.01$ . (E) Guide cannula was coated with fluorescent dye to  
127 assess placement (Left) relative to desired location in anterior BLA (Right; bregma -1.4 mm; Allen  
128 brain atlas). Note that the injection cannula extended 0.5 mm further into the BLA.

129 treatment effects and a significant interaction between the two (Figure 1B), and post-hoc  
130 analysis revealed significant reduction in the consumption of saccharin (CTA vs. Test) for  
131 the vehicle group, indicating impairment of learning for the actinomycin-D treated group  
132 compared to control mice. As a convergent measure, we also assessed the strength of  
133 CTA memory by calculating the relative consumption of saccharin during the test day to  
134 that consumed on the training day (Neseliler et al., 2011). The differences between the  
135 groups were large (23% in the vehicle group vs. 80 % for the actinomycin D group) and

136 significant. Meanwhile, actinomycin-treated mice were neither impaired in their ability to  
137 detect the palatability of saccharin, nor in their drinking behavior—consumption of  
138 saccharin during CTA training was similar for the two groups, as was consumption of  
139 water 8 hours after the test (Figure 1E), suggesting that these nonspecific effects cannot  
140 account for the memory impairment. Thus, BLA transcription is essential for CTA memory  
141 formation. These results extend prior work showing the importance of BLA protein  
142 synthesis for CTA memory (Josselyn et al., 2004) and together show that training induces  
143 both transcription and translation important for CTA in the BLA.

144

#### 145 **Stk11 expression is regulated in BLA projection neurons following CTA**

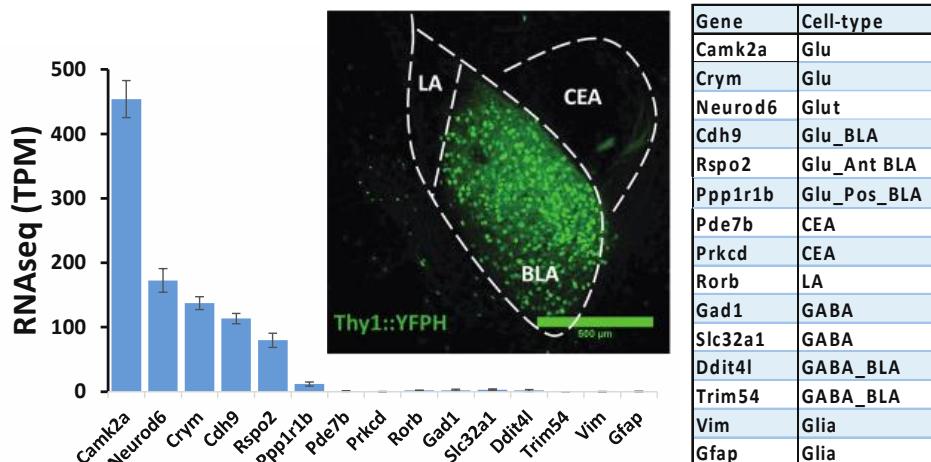
146 In order to identify specific transcripts that might be necessary for CTA learning within the  
147 BLA, we used cell type-specific RNA-seq to profile transcriptional changes in sorted BLA  
148 projection neurons (BLApn). We manually isolated fluorescently labeled BLApn neurons  
149 from YFP-H mice (Feng et al., 2000) in which YFP is expressed under the Thy1 promoter  
150 in a large population of excitatory projection neurons located in the anterior part of the  
151 nucleus (Sugino et al., 2006; Jasnow et al., 2013; McCullough et al., 2016). RNA  
152 sequencing was performed separately on YFP<sup>+</sup> BLApn harvested 4 hours following  
153 training from mice undergoing CTA, and from taste-only controls (n=4/group) (Figure 2  
154 and Table 1). Sequencing results (Figure 2A) also confirmed the purity and molecular  
155 identity of YFP-H neurons in the BLA, as transcripts known to be expressed in BLApn  
156 and other forebrain excitatory projection neurons were enriched and transcripts known to  
157 be expressed in inhibitory interneurons, glia cells and other neurons in the vicinity of the  
158 BLA (lateral amygdala or central amygdala) were virtually absent. Moreover, this  
159 observed pattern of expression was comparable to that reported in other studies profiling  
160 the same population (Sugino et al., 2006; McCullough et al., 2016).

161

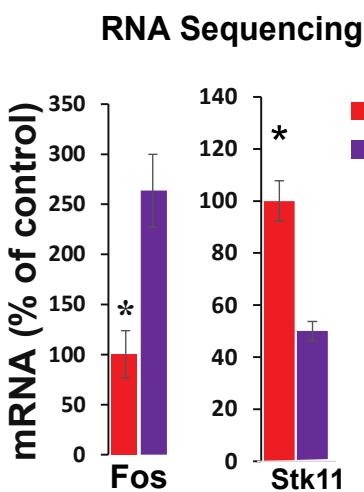
162 CTA training changed expression of many genes. Table 1 lists the 20 transcripts showing  
163 differential expression between CTA and taste control group based on robust expression  
164 criteria:  $2 \leq \text{fold change} \leq 0.5$ ,  $p < 0.01$  (unpaired t-test), transcripts-per-million (TPM)  $\geq 30$ .  
165 Included among these transcripts is the activity dependent transcription factor, Fos which  
166 has previously been shown to be upregulated in BLApn following CTA and other learning  
167 paradigms (Zhang et al., 2002; Yasoshima et al., 2006; Mayford and Reijmers. 2015;  
168 Uematsu et al. 2015). Our screen for differentially expressed genes also identified Stk11  
169 (also known as LKB1; Figure 2B) a kinase well studied in the context of cancer, cell  
170 growth and development, but not previously known to be involved in learning and  
171 neuronal plasticity (Bardeesy et al., 2002; Alessi et al., 2006; Barnes et al., 2007;  
172 Gurumurthy et al., 2010; Courchet et al., 2013). Changes in the expression of both Fos  
173 and Stk11 were validated by qPCR in separate experiments, which revealed a 4.6-fold  
174 increase in Fos mRNA ( $t(6)=2.5$ ;  $p=0.045$ ) and a 1.9-fold decrease in Stk11 ( $t(6)=-2.5$ ;  
175  $p=0.046$ ) (Figure 2C). To further examine the significance of these transcriptional  
176 changes we also analyzed the levels of Fos and Stk11 protein in the BLA. Due to the  
177 availability of antibodies to Fos suitable for immunohistochemistry, we measured the

178 fraction of Fos-expressing YFP-H neurons in the BLA 4 hours following CTA and found a  
 179 significant increase relative to lithium chloride-only and taste-only control groups (Figure  
 180 2-figure supplement 1). Lacking an antibody to Stk11 usable for immunostaining of brain  
 181 sections, we examined Stk11 protein 4 hours following CTA and taste control conditions

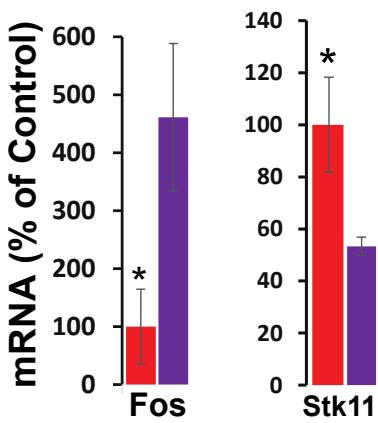
**A)**



**B)**



**C)** **qPCR Validation**



182  
 183 **Figure 2. RNA sequencing from BLApn 4 hours following CTA training.** (A) BLApn were  
 184 isolated from YFP-H mice following CTA or taste control (N=4/group). Neurons (150-200) were  
 185 manually sorted from coronal slices (LA-lateral amygdala; CEA-central amygdala). Abundant  
 186 transcripts (histogram, averaged across both groups) are enriched for those expected in the  
 187 population and depleted for those expressed in other nearby populations (table) including  
 188 GABAergic interneurons, glia, neurons in LA or CEA; Sugino et al., 2006; Kim et al., 2016; Allen  
 189 brain atlas). Glu- GABA-, glutamatergic, GABAergic neurons; AntBLA, PostBLA- Anterior and  
 190 posterior portions of the BLA. TPM- transcript per million. (B) Among genes meeting robust criteria  
 191 for differential expression (see table 1) Stk11 and Fos were selected for further analysis, including  
 192 qPCR confirmation (C) in separate experiments (N=4/group; \*p<0.05).

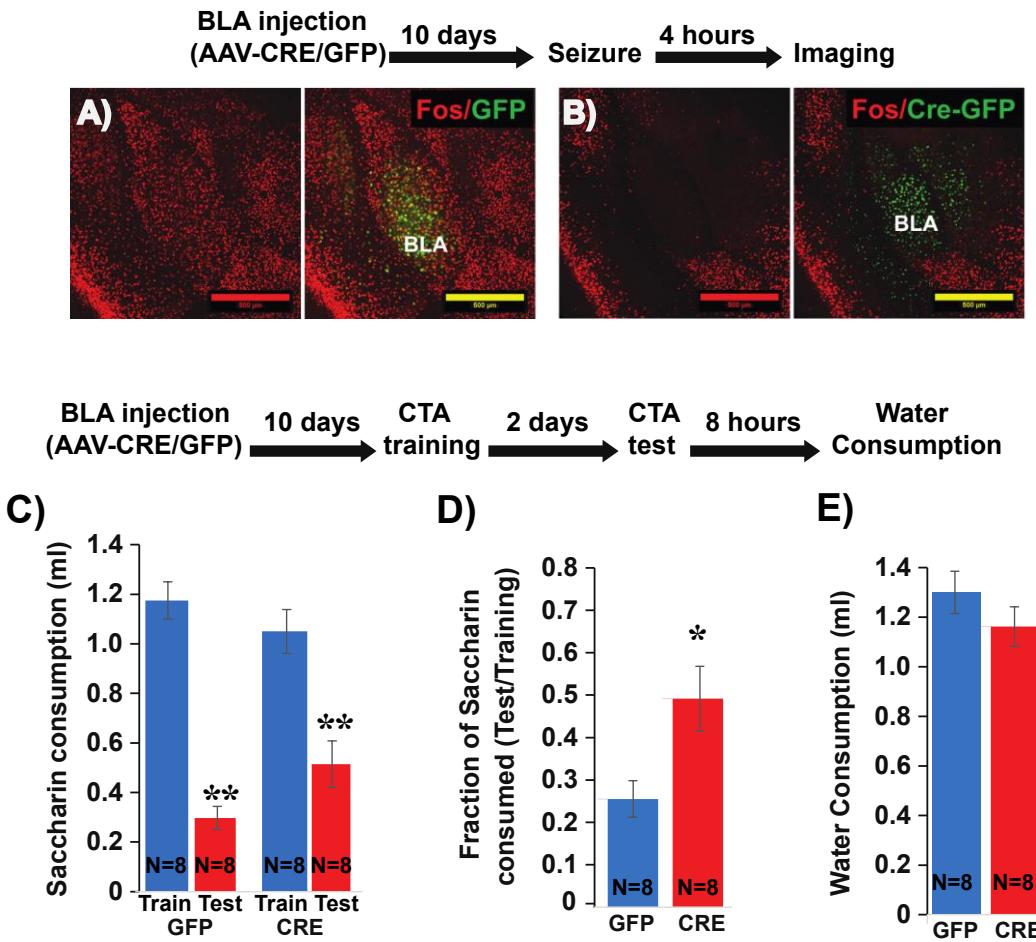
193 using immunoblotting of proteins isolated from the anterior BLA. Surprisingly, we found a  
194 1.8- fold increase in Stk11 protein (Figure 2-figure supplement 2). This confirms the fact  
195 that Stk11 expression is altered following CTA, but suggests complexity in the dynamics  
196 of the expression and potential mismatch in the timing or magnitude of changes in  
197 transcript and protein.

198  
199 Stk11 is at the apex of the AMP-related kinase pathway and mediates its effects by  
200 phosphorylating one or more of 13 different downstream kinases, all of which share some  
201 homology with AMP-kinase (Lizcano et al., 2004; Shackelford and Shaw. 2009). Among  
202 these, YFP<sup>+</sup> BLApn had moderate expression of Brsk2 (TPM=39.9) and Mark2/3  
203 (TPM=59.3 and 73.7 respectively; Figure 2-figure supplement 3), which have known roles  
204 in establishing cell polarity during neuronal development (Barnes et al., 2007; Shackelford  
205 and Shaw. 2009). Expression of AMP kinase itself (Prkaa1/2), an important metabolic  
206 regulator in many cell types (Shackelford and Shaw. 2009) was lower (TPM=16.4 and  
207 22.3 respectively). Comparing kinase expression between CTA and control groups  
208 revealed nearly two-fold less expression of Mark2 following CTA (fold-change=0.41;  
209 p=0.04, unpaired t-test; data not shown) but this was not significant after Bonferroni  
210 correction across the compared kinases.

211 We also analyzed the impact of CTA on the transcriptional profile of layer 5 pyramidal  
212 neurons labeled in strain YFP-H and Pvalb-expressing inhibitory interneurons in the GC,  
213 a brain region in which transcription is also known to be important for CTA learning  
214 (Imberg et al., 2016). RNA sequencing was performed on YFP<sup>+</sup> and Pvalb<sup>+</sup> neurons in  
215 the GC harvested from mice 4 hours following CTA training, and from taste-only controls  
216 (n=3/4/group). Table 2 and 3 lists the transcripts showing the most differentially expressed  
217 genes using the same criteria used in the BLA: 2 ≤ fold change ≤ 0.5, p<0.01 (unpaired  
218 t-test), transcripts-per-million (TPM) ≥ 30. While Pvalb<sup>+</sup> neurons showed a robust  
219 transcriptional response, evident by 19 genes reaching the criteria, only one gene  
220 reached the same criteria in YFP<sup>+</sup>, suggesting a weaker CTA-driven transcriptional  
221 response in these neurons. Importantly, the expression of Fos and Stk11 in both YFP<sup>+</sup>  
222 and Pvalb<sup>+</sup> neurons, did not differ between CTA and control groups (Figure 2-figure  
223 supplement 4 and Table 2 and 3). This data suggest that the differential expression of  
224 Fos and Stk11 during CTA learning may be specific to a subset of cell-types within the  
225 circuit.

226  
227 **Fos and Stk11 expression in BLA projection neurons are necessary for memory  
228 formation**

229 We next wished to determine whether any of the transcriptional changes in BLAps that  
230 correlate with learning are indeed necessary for learning to occur. Since both Fos and  
231 Stk11 protein increase following CTA, we pursued a loss of function (LOF) strategy. To  
232 restrict LOF to BLAps, we performed conditional deletion by injecting Cre recombinase  
233 into mice carrying alleles of Fos (Zhang et al., 2002) or Stk11(Nakada et al., 2010) in



234  
235 **Figure 3. Deletion of Fos from BLA<sup>pn</sup> reduces the strength of learning.** (A,B) BLA of Fos<sup>f/f</sup>  
236 mice were infected with viruses expressing Cre-GFP (B) or GFP alone (A). Fos induction was  
237 tested 10 days later, 4 hours after onset of seizures in response to kainic acid (20 mg/kg). Cre  
238 injected BLA's had reduced Fos expression confirming penetrance of the knock-out. (C-E)  
239 Fos deletion from BLA<sup>pn</sup> attenuates CTA learning. Fos f/f mice received Cre and control viruses  
240 bilaterally and were trained for CTA 10 days later and then tested after an additional 48 hours.  
241 (C) Both groups exhibited significant memory reflected by reduced saccharin consumption  
242 between training and testing sessions. Two-way ANOVA revealed a significant effect of training  
243 ( $F(1,28)=63.06$ ;  $p=1.15E-8$ ), but not of genotype ( $F(1,28)=0.261$ ;  $p=0.614$ ), although there was a  
244 significant interaction ( $F(1,28)=4.9$ ;  $p=0.03$ ). Post hoc analysis (Bonferroni) revealed that both  
245 GFP (N=8) and Cre (N=8) group reductions following CTA (test vs. train) were significant (GFP:  
246  $p=7.17E-8$ ; Cre:  $p=3.47E-4$ ) but differences between other groups were not. (D) Fos deletion from  
247 BLA<sup>pn</sup> reduced memory strength measured as the fraction of saccharin consumed (test/training):  
248 25% (GFP) versus 49% (Cre) and this difference in ratios was significant ( $t(14)=-2.697$ ;  $p=0.017$ ).  
249 (E) Reduced saccharin consumption cannot be attributed to overall inhibition of drinking as the  
250 amount of water drunk 8 hours later did not differ ( $p=0.26$ ). \* $p<0.05$ ; \*\* $p<0.01$ .

251 which key exons are flanked by lox-p sites. In both cases, recombination leads to a  
252 functionally null allele and analyses were carried out in homozygous (<sup>f/f</sup>) animals. Cre was  
253 delivered by injecting AAV2/5-Camk2α::Cre-GFP into the BLA bilaterally. As expected,

254 this led to GFP expression in excitatory projection neurons, but not in BLA interneurons  
255 or adjacent GABAergic neurons in the Central Amygdala (CEA; Figure 3,5). Animals  
256 carrying the same genotypes but receiving AAV2/5-Camk2α::GFP served as controls.  
257 Injections were performed 10 days prior to analysis to allow time for LOF to occur.  
258

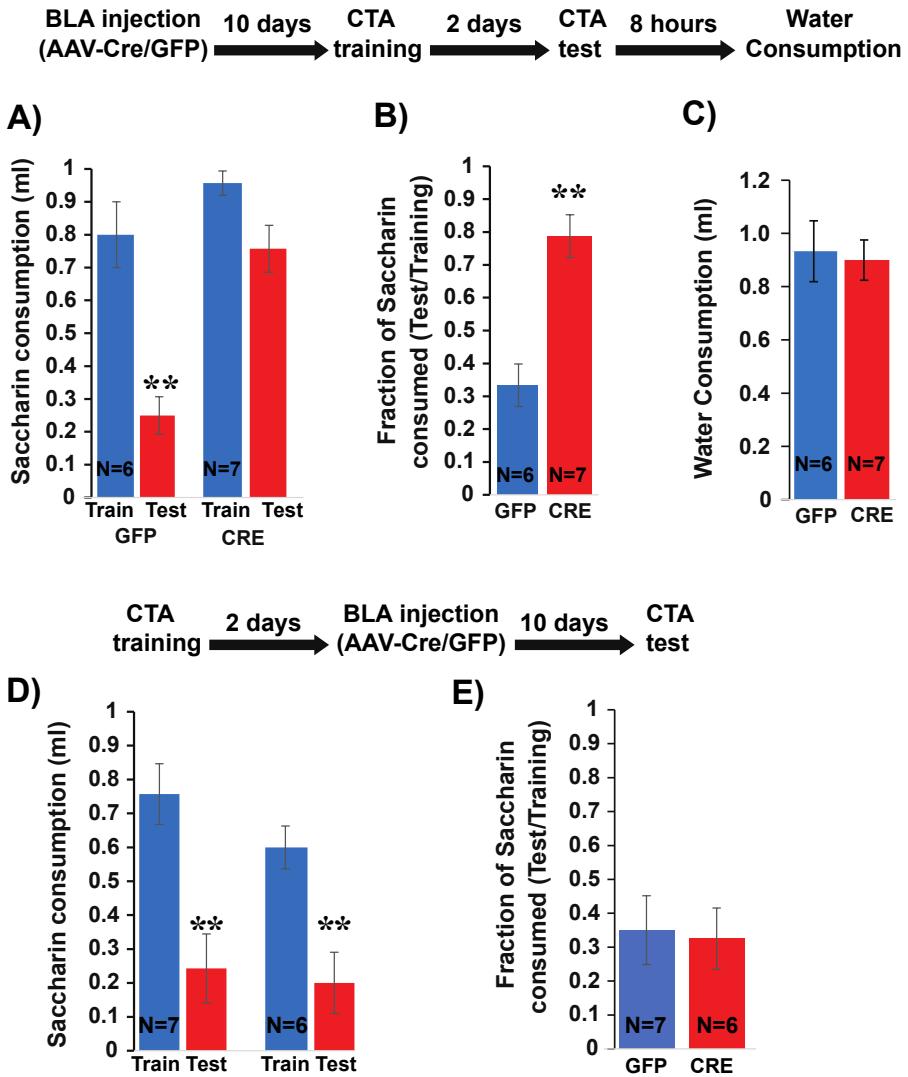
259 To assess the efficacy of this approach for CTA learning, we first examined the necessity  
260 of Fos expression. Fos is known to contribute to multiple forms of learning and plasticity  
261 (Zhang et al., 2002; Tonegawa et al., 2015) and has previously been implicated in CTA  
262 (Lamprecht and Dudai. 1996; Yasoshima et al., 2006). To first confirm effective Cre-  
263 mediated recombination, we tested the ability of viral Cre to prevent widespread Fos  
264 expression in the BLA immediately following kainic acid-induced seizures. Fos staining  
265 performed four hours after seizures revealed strong induction of Fos protein throughout  
266 the BLA of control mice injected with the control virus, and diminished Fos expression in  
267 mice injected with Cre (Figure 3A,B). We then tested the effect of Fos deletion on CTA in  
268 separate animals. Cre-GFP and GFP control AAV's were injected into the BLA of Fos<sup>ff</sup>  
269 mice, CTA training occurred 10 days later, and long-term memory was tested after an  
270 additional 2 days.

271 The results show that while both groups could form CTA memory, Cre injected mice  
272 showed significantly weaker memory (Figure 3C-E). This confirms our ability to  
273 manipulate memory by conditional knockout in BLA<sub>pn</sub> neurons. The results refine those  
274 of a prior study using antisense injections and germline knockouts (Yasoshima et al.,  
275 2006).

276 Next, we used the same strategy to test the necessity of Stk11 expression in BLA<sub>pn</sub> for  
277 CTA memory. BLA of Stk11<sup>ff</sup> mice were injected bilaterally with AAV expressing Cre-  
278 GFP, or GFP alone. Ten days later both groups of mice were trained for CTA and tested  
279 after an additional two days. The results revealed a near complete loss of learning in the  
280 Stk11 KO mice (Figure 4A,B), which exhibited no significant reduction in the amount or  
281 ratio of saccharin consumed after training. Control mice exhibited significant reductions  
282 consistent with a similar degree of learning to that seen in previous control experiments.  
283 The differences in the consumption of saccharin during the test day were not attributable  
284 to overall reduced drinking, as both group of animals consumed comparable amounts of  
285 water 8 hours later (Figure 4C). Taken together, these results indicate that Stk11  
286 expression in BLA<sub>pn</sub> is essential for CTA memory.  
287

288 Stk11 deletion prior to CTA training can potentially alter multiple memory stages including  
289 memory formation and retrieval (Levitin et al., 2016b). Because CTA memory is long-  
290 lasting after even a single training session, it is possible to distinguish an effect of Stk11  
291 deletion on memory formation from an effect on retrieval by performing the deletion  
292 immediately after training and before testing. We performed the same knockout and  
293 control experiments as those described above, but altered our protocol so that Cre and  
294 control viruses were injected 2 days following CTA training. Testing occurred 10 days  
295 later, allowing the same period for Cre expression and recombination to occur. Since the  
296 memory was being tested after a longer period (twelve days vs. two days), we used a

297 stronger version of the CTA protocol (I.P injection of 2% LiCl instead of 1%; Figure 4-



298  
299 **Figure 4. Deletion of Stk11 from BLApcn impairs CTA learning.** (A) Stk11<sup>f/f</sup> mice were infected  
300 with Cre or control viruses 10 days before CTA training and were tested 48 hours later. Despite  
301 significant reduction in saccharin consumption between testing and training sessions in control  
302 mice, Stk11 KO animals showed almost no learning. Two-way ANOVA revealed significant group  
303 and genotype effects (groups:  $F(3,22)=19.29$ ;  $p=0.00002$ ; genotype:  $F(1,22)=23.43$ ;  $p=7E-5$ ; as  
304 well as a significant interaction:  $F(1,22)=6.5$ ;  $p=0.018$ ). Post hoc analysis confirmed that GFP  
305 injected mice ( $N=6$ ) developed strong CTA indicated by the significant reductions ( $p=0.0001$ ) of  
306 saccharin consumption, but Cre injected mice ( $N=7$ ) failed to significantly reduce their  
307 consumption ( $p=0.259$ ). (B) GFP controls consumed only 33% during the test, relative to training,  
308 but KO mice consumed 78% and this difference was highly significant ( $t(11)=-4.91$ ;  $p=4E-4$ ). (C)  
309 Reduced saccharin consumption does not reflect overall reduction in drinking measured 8 hours  
310 later ( $t(11)=0.25$ ;  $p=0.87$ ). \*\* $p<0.01$ . (D) Stk11 deletion after long-term memory formation has no  
311 effect on CTA retention. Stk11 f/f mice received Cre and control viruses 2 days after CTA training  
312 and were tested 10 days later. Two-way ANOVA revealed a significant effect of training

313 (F(1,22)=21.06; p=8E-6) but no significant effect of genotype (F(1,22)=1.9, p=0.17) or interaction  
314 (F(1,22)=0.63, p=0.43). Post hoc analysis confirmed significant reductions in both groups  
315 following CTA (GFP: N=7, p=1E-4. Cre: N=6, p=0.006). (E) There was no significant difference in  
316 CTA intensity as measured by the fraction of saccharin consumed (t=-0.18, p=0.861). \*\*p<0.01.  
317 figure supplement 1).

318  
319 Both Cre-GFP and GFP injected mice developed CTA (Figure 4D) – there was no  
320 significant between-group difference in the intensity of memory, assessed from the ratio  
321 of saccharin consumed during the test (Figure 4E). Since the same deletion produces a  
322 profound effect when occurring prior to training, this suggests that deletion of Stk11 from  
323 BLApn does not affect the retention and retrieval of CTA memory, provided memory was  
324 already formed prior to performing the knockout. This argues that Stk11 is required for  
325 CTA memory formation.

326  
327 **Stk11 deletion does not impact basal aspects of taste behavior**

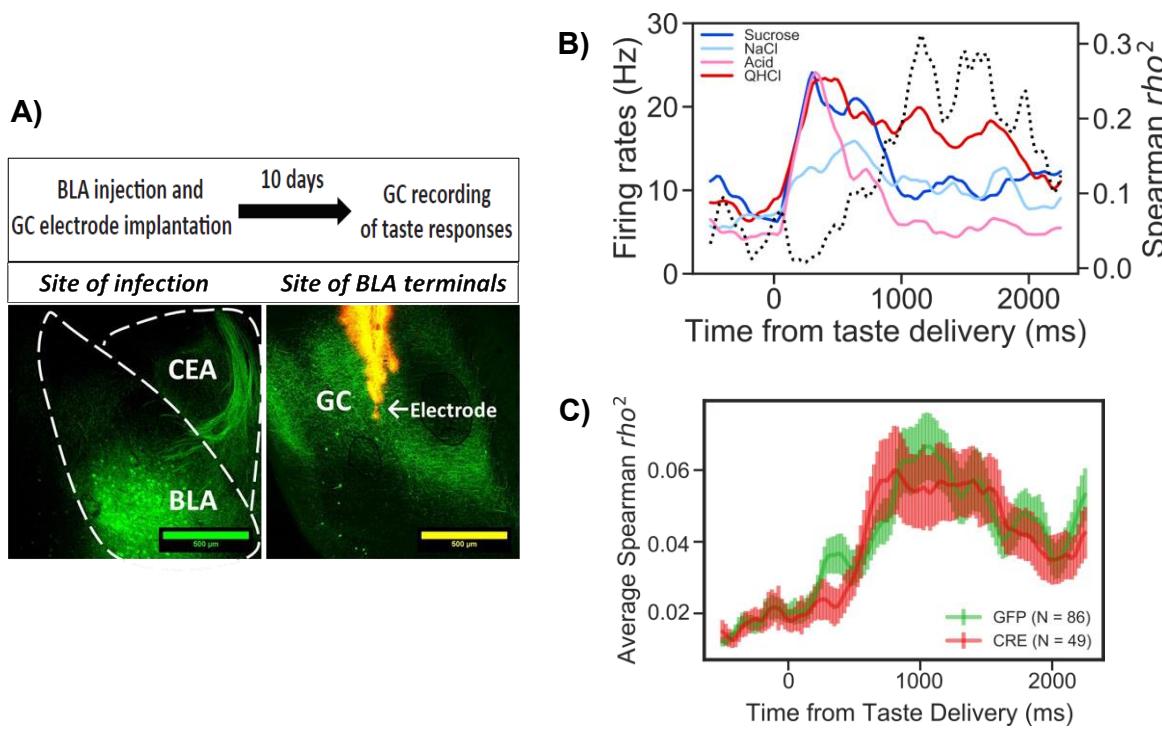
328 Since Stk11 deletion must occur prior to CTA training to have an effect on learning, we  
329 needed to rule out the possibility that its effect on memory came via disruption of either  
330 the responsiveness of BLA neurons to training stimuli, or the output of BLA neurons,  
331 which is known to be required for palatability coding within GC.

332 To assess the responsiveness of BLA neurons to training stimuli, we asked whether  
333 training would still activate Fos expression in BLAps after Stk11 deletion. As shown in  
334 (Figure 5- figure supplement 1), there is no significant difference between the number of  
335 Fos+ neurons following Cre injections and control GFP injections (N=2/group; p<0.05, t-  
336 test).

337 A more rigorous test of BLA function is to determine whether palatability coding in the GC  
338 is intact following knockout, since this is known to depend on intact output from the BLA  
339 (Piette et al., 2012; Samuelsen et al., 2012; Lin and Reilly. 2012; Lin et al., 2018). If Stk11  
340 deletion disrupts gustatory activation of BLAps or their output to the GC, palatability  
341 coding recorded in the GC should be impaired. To test this, Stk11 deletion in BLAps was  
342 performed as before and 10 days later multi-channel *in vivo* recordings of GC taste  
343 responses were obtained. Recordings were targeted to the ventral part of GC, since BLA  
344 projects to these regions (Figure 5A; Haley et al., 2016; Levitan et al., 2019). Palatability  
345 coding was assessed with a battery of four tastes with hedonic values ranging from  
346 palatable (sucrose and sodium-chloride) to aversive (citric-acid and quinine; for details  
347 see Levitan et al., 2019).

348 Figure 5 shows the results of GC taste responses following Stk11 deletions in BLA. Figure  
349 5B illustrates the peri-stimulus histogram of a representative GC neuron responding to  
350 the taste battery. As observed previously in rats and mice (Katz et al., 2001; Sadacca et  
351 al., 2013; Levitan et al., 2019), different aspects of taste processing are encoded in firing  
352 rates sequentially. In the first five hundred milliseconds or so post-taste delivery, neurons  
353 show different firing rates to different tastes (i.e., reflecting taste identity coding), while

354 later in the responses, differential response rates reflect the hedonic values of tastes (i.e.,  
355 taste palatability coding). These properties were maintained in the mice studied here: as  
356 indicated by the dashed line, the magnitude of the correlation between the neuron's  
357 stimulus evoked firing rates and the behaviorally-determined palatability ranking rose  
358 significantly only after the first half a second following taste delivery. The averaged  
359 correlation magnitudes across neurons from Cre and GFP injected mice are shown in  
360 Figure 5C. Inspection of the figure suggests that BLA Stk11 deletion had little effects on  
361 GC taste palatability coding; the correlations in both GFP- and Cre-injected groups rise  
362 around half a second and peaks at about one second after taste delivery. An ANOVA  
363 found no significant group differences across each time bin, suggesting that Stk11  
364 deletion in BLA has little detectable influence on GC taste processing.



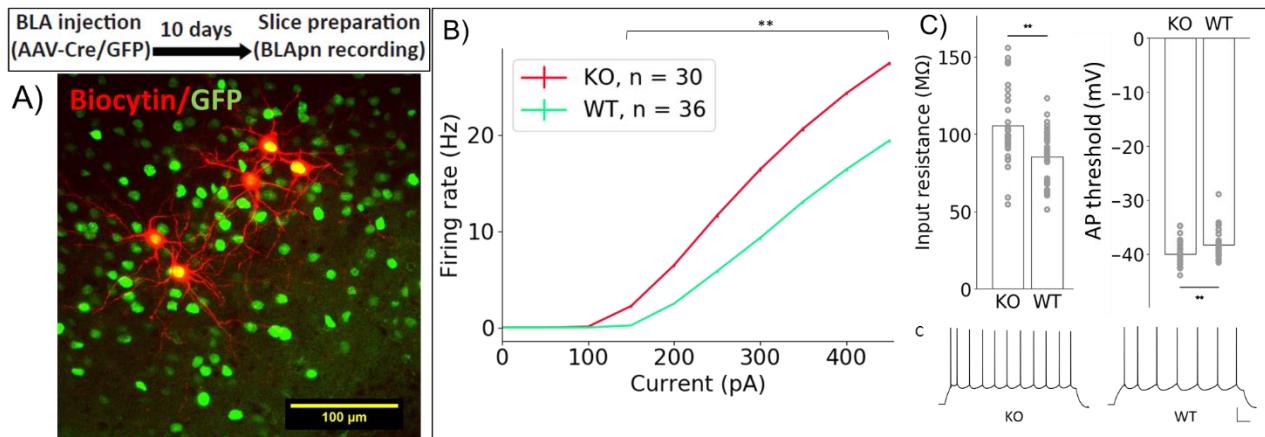
365  
366 **Figure 5. Stk11 deletion in BLA does not affect taste palatability coding in the GC. (A)**  
367 The BLA of  $Stk11^{tf}$  mice was infected bilaterally with Cre or control viruses. The ventral GC, where  
368 BLA projections terminate (Haley et al., 2016) was implanted with a multi-electrode array 10 days  
369 later to record GC taste responses to a battery of four tastes differing in their hedonic value, the  
370 palatable sucrose and sodium-chloride and the aversive citric acid and quinine (Levitin et al.,  
371 2019). Images show BLA injection site (left), and labeled BLA terminals in the ventral GC co-  
372 localized with the site of dye-labeled electrodes (right). **(B)** PSTHs (colored lines) from a  
373 representative GC neuron in a GFP-injected control mouse that responded significantly to all  
374 tastes. Dashed line represents the magnitude of correlation between firing rates and behaviorally  
375 measured palatability. **(C)** Correlation coefficients averaged across all recorded units in  
376 GFP(control) and Cre- injected mice. As revealed in a 2-way ANOVA, palatability correlations in  
377 both groups rise steeply between 800-1000 ms with no significant difference between genotypes  
378 ( $F(1,133)=0.13$ ,  $p = 0.72$ ) or interaction ( $p = 0.99$ ).

379 Taken together, our molecular and electrophysiological analyses suggest that the  
380 memory deficit observed after *Stk11* deletion is unlikely to be due to a deficit in basic taste  
381 processing. Rather, *Stk11* deletion likely impairs memory by affecting the process of  
382 memory formation.

383 **Stk11 or Fos deletion and CTA produce opposing effects on BLA intrinsic  
384 excitability**

385 What cellular mechanisms mediate the effects of Fos and *Stk11* on memory formation?  
386 Obvious candidates abound: for instance, long-term memory is known to be accompanied  
387 by changes in both the intrinsic excitability of neurons (Zhang and Linden, 2003;  
388 Mozzachiodi and Byrne, 2010) and in the strength of their synaptic connections. Recent  
389 studies have shown that the intrinsic excitability of BLApn can be modulated bi-  
390 directionally during reinforcement learning, with positive reinforcement leading to  
391 increased and negative reinforcement to decreased intrinsic excitability (Motanis et al.,  
392 2014).

393 To determine whether *Stk11* might influence memory formation by altering intrinsic  
394 excitability, we compared the excitability of BLApn recorded in ex-vivo slices from mutant  
395 animals receiving Cre-GFP (Figure 6). The results reveal a marked increase in the  
396 intrinsic excitability of BLApn following deletion of *Stk11*, relative to GFP-only controls.  
397 Cre infected neurons had higher firing rates than GFP



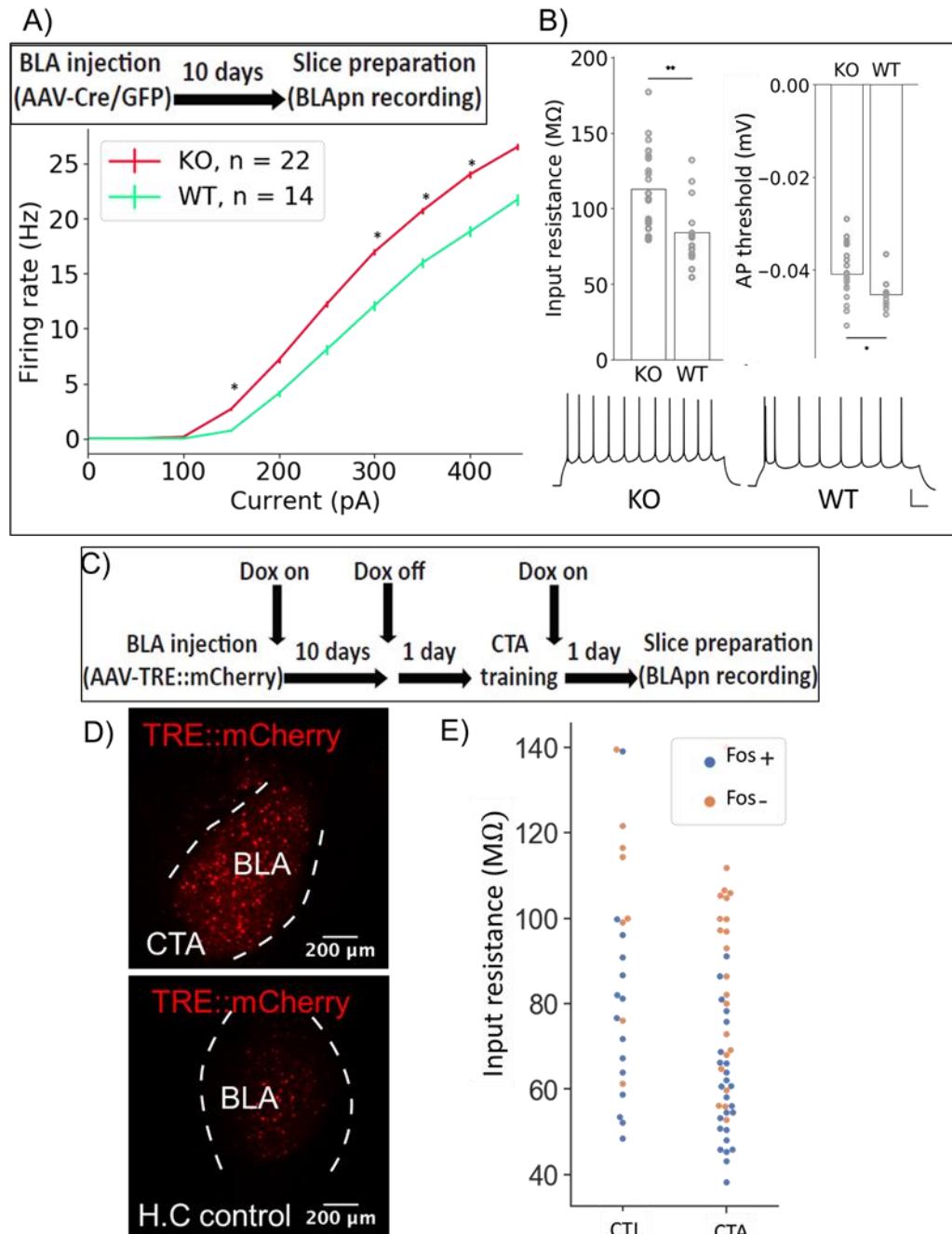
398

399 **Figure 6. *Stk11* deletion in BLApn increases intrinsic excitability.** (A) Whole-cell recordings  
400 obtained from BLApn in ex vivo slices of *Stk11*<sup>fl/fl</sup> mice 10 days after injection of Cre or control  
401 virus. *Stk11* neurons were targeted based on GFP expression and validated post hoc based on  
402 Biocytin fills. (B) Firing rates plotted against input current (F-I). Error bars (SEM) are too small to  
403 be seen for some points. Less current is needed to evoke firing in *Stk11*-KO neurons compared  
404 to *Stk11*-WT neurons (interpolated rheobase,  $F(1,64) = 10.63$ ,  $p = 1.8e-3$ ). The F-I slope is larger  
405 for *Stk11* knockout neurons ( $F(1,64) = 23.47$ ,  $p = 8.4e-6$ ). (C) *Stk11*-KO neurons have increased  
406 input resistance ( $F = 14.41$ ,  $p = 3.3e-4$ ) and decreased threshold for generating action potential  
407 ( $F = 7.68$ ,  $p = 7.3e-3$ ). Traces are sample responses to 300 pA current steps. Scale bar: 100 ms,  
408 20 mV.

409 neurons for any given amount of current input resulting in a steeper slope of the firing rate  
410 vs. current (F-I) curve. Threshold firing was initiated at a lower level of current injection  
411 (i.e. the rheobase was lower; Figure 6C). This reflected a higher resting input resistance  
412 and a slightly lower voltage threshold. Some other electrophysiological properties also  
413 differed (see Table 4) including sag ratios, action potential amplitudes, and the medium  
414 and slow afterhyperpolarizations, while others, such as the degree of firing rate  
415 accommodation, spike widths and resting membrane potentials did not. Thus, Stk11  
416 deletion from BLApn neurons increases overall intrinsic excitability, most likely by  
417 affecting multiple biophysical properties of these neurons.

418  
419 Since Stk11 and Fos deletion both impair CTA learning, we wondered whether Fos  
420 deletion also increases the intrinsic excitability of BLApn. Analysis of Fos KO neurons  
421 revealed a similar increase in firing relative to control neurons (N for: WT=14, KO=22;  
422 Figure 7A-B). Two-way ANOVA indicated a significant effect of genotype (in addition to  
423 the expected effect of current level). Significant differences in input resistance and action  
424 potential threshold were also detected.

425 Finally, we asked whether we could detect an effect of learning itself on neuronal  
426 excitability in the BLA. A difficulty with this experiment is that learning may have different  
427 effects on different populations of BLApn as evidenced by the fact that some neurons  
428 increase Fos expression following training, while others do not (Figure 2-figure  
429 supplement 2). In order to separately examine these two populations following training,  
430 we made use of the reporter system developed by Reijmers and colleagues, in which  
431 elements of the Fos promoter are used to drive the tet transactivator (tTA) which can then  
432 prolong and amplify expression of a reporter marking Fos-activated cells, when  
433 Doxycycline (Dox) is absent (Reijmers et al., 2007). In this case, we provided the tet-  
434 dependent reporter via an AAV (TRE::mCherry) injected into the BLA ten days prior to  
435 training. Animals were fed Dox until 24 hours prior to training to limit background activation  
436 (Figure 7C). Control experiments showed that CTA training resulted in a greater number  
437 of neurons expressing Fos 24 hours after training, compared to home cage controls  
438 (Figure 7D). Twenty-four hours following CTA training or taste-only control experiments,  
439 recordings were obtained from BLApn in acute slices. Two-way ANOVA revealed that  
440 BLApn in the CTA animals have lower input resistance than those in control animals and  
441 that Fos-activated neurons have lower input resistance than unlabeled neurons (Figure  
442 7E). Notably, this change is in the opposite direction from that produced by deletions of  
443 Stk11 and Fos, two manipulations that impair learning. This suggests that these mutations  
444 may interfere with learning by impairing changes in intrinsic excitability that are required  
445 for learning to occur.



446

447 **Figure 7. Fos deletion and CTA have opposing effects on resting input resistance. (A,B)**  
448 Whole-cell patch-clamp recordings obtained from BLA<sub>pn</sub> in ex vivo slices of Fos<sup>fl/fl</sup> mice 10 days  
449 after injection of Cre or control virus. Fos-KO neurons exhibit increased firing in response to  
450 current injection compared to Fos-WT neurons. **(A)** Average frequency-current (FI) curves. Two-  
451 way mixed ANOVA revealed a significant difference in firing  $F(1,34) = 4.213$ ,  $p = 0.045$ . **(B)**  
452 Significant differences in input resistance ( $F(1) = 10.62$ ,  $p = 2.5e-3$ ) and threshold for generating  
453 action potential ( $F(1) = 6.82$ ,  $p = 0.013$ ) between Fos-KO and Fos WT neurons were also  
454 detected. Sample responses to 250 pA current steps. Scale bar: 100 ms, 20 mV. **(C)** Tet-  
455 dependent labeling of Fos expressing neurons (Reijmers et al., 2007) during CTA training.

456 Fos::tTa mice were injected with AAV-TRE::mCherry and received food with 40 ppm Doxycycline  
457 (Dox) to suppress reporter expression. One day prior to CTA training, Dox was removed. Acute  
458 slices were prepared 24 hours following CTA (Saccharin+lithium) or control (Saccharin+saline)  
459 training. **(D)** Fos expressing neurons labeled through Tet-off system with mCherry fluorescent  
460 reporters in the BLA. **(E)** Input resistances of Fos<sup>+/−</sup> neurons in the BLA, following CTA training  
461 and taste-only control experiments. Two-way ANOVA reveals that neurons from CTA animals  
462 have lower input resistance than those from control animals ( $F(1,65) = 10.26, p = 2.1e-3$ ) and that  
463 Fos<sup>+</sup> neurons have lower input resistance than Fos<sup>−</sup> neurons ( $F(1,65) = 23.64, p = 7.5e-6$ ). Post-  
464 hoc tests show that among Fos<sup>+</sup> neurons, neurons in CTA animals have lower input resistance ( $p$   
465 = 0.015), while among Fos<sup>−</sup> neurons, there was no significant difference between CTA and control  
466 animals ( $p = 0.1$ ). Differences between Fos<sup>+</sup> and Fos<sup>−</sup> neurons did not reach post-hoc significance  
467 in either the CTA ( $p = 0.25$ ) or control animals ( $p = 0.12$ ) considered alone.

468

## 469 **Discussion**

470 This study is the first to identify a role for Stk11, a master kinase at the top of the AMP-  
471 related kinase pathway, in long-term memory. Using CTA as a behavioral paradigm, we  
472 first established a causal requirement for transcription in BLApn during establishment of  
473 long-term memory, and went on to show that changes in Stk11 transcription and  
474 translation accompany CTA learning. Cell-type specific conditional knock-out of Stk11 in  
475 BLApn revealed it to be necessary for CTA memory formation but not for retrieval, once  
476 memories were established. Slice recordings revealed that Stk11 modulated the intrinsic  
477 excitability of these neurons and further investigations suggested the general importance  
478 of excitability changes for memory—deletion of the immediate early gene Fos in BLApn  
479 altered excitability similarly to Stk11 deletion, and conversely activation of Fos during  
480 learning reduced excitability.

## 481 **BLA projection neurons undergo transcription important for CTA learning**

482 It is well established that BLA neurons play a necessary role in CTA learning. Multiple  
483 studies confirm that activity in the BLA is required for memory formation and retrieval  
484 (Yasoshima et al., 2000; Ferreira et al., 2005; Garcia-de la Torre et al., 2014; Molero-  
485 Chamizo, et al., 2017). CTA also requires protein synthesis in the BLA (Josselyn et al.,  
486 2004), but whether new transcription is also required, and if so, the identities of the  
487 required transcripts and the cellular processes they promote were not previously known.

488 Here we show that BLA projection neurons (BLApn) undergo transcriptional changes  
489 important for CTA memory. Inhibiting transcription during CTA training impairs memory  
490 tested 48 hours later. Using cell-type specific RNA sequencing, we go beyond this simple  
491 insight to identify the transcripts that are altered in expression in BLApn four hours after  
492 pairing of the conditioned and unconditioned stimuli. For comparison, we also examined  
493 changes in transcript levels in pyramidal neurons and parvalbumin-positive interneurons  
494 in gustatory cortex. These profiling experiments provide a resource for future  
495 investigations of other molecules potentially involved in CTA in BLA and GC.

496 Perhaps the strongest case for new transcription in BLApn involved in learning can be  
497 made for the immediate early gene Fos. It is well known that Fos transcription and  
498 translation are activated in the forebrain by a variety of memory paradigms (Mayford and  
499 Reijmers. 2015), and more specifically by CTA in BLApn (Uematsu et al., 2015). The  
500 YFP-H neurons studied here include the majority of BLApn in the anterior portion of the  
501 nucleus (Feng et al., 2000; Sugino et al., 2006; Jasnow et al., 2013; McCullough et al.,  
502 2016) and the fact that many of these neurons express Fos protein (Figure 2-figure  
503 supplement 1) and project to GC (Figure 5A and Haley et al., 2016) supports the  
504 suggestion that they are among the population of BLApn transcriptionally activated by  
505 training and participating in the BLA-GC circuit implicated in learning by prior studies  
506 (Grossman et al., 2008). Since Fos transcript and protein are short-lived (Spiegel et al.,  
507 2014; Chowdhury and Caroni. 2018) the most parsimonious explanation is that training  
508 induces new transcription and translation, and that it is these effects that are disrupted by  
509 the Fos KO in BLApn (Figure 3). Nevertheless, even for Fos, we cannot rule out the  
510 possibility that effects of the knockout preceding training, such as altered excitability, are  
511 what are necessary for learning, rather than new transcription and translation immediately  
512 following learning. Resolution of this issue will require new technologies like protein  
513 knockout (Clift et al., 2017) with temporal resolutions measured in minutes rather than  
514 days.

515 The results of selectively knocking Fos out in BLApn clarify the results of earlier studies  
516 in which Fos was manipulated with infusion of antisense oligonucleotides (Lamprecht et  
517 al., 1996; Yasoshima et al., 2006) or via global knockout (which had no effect on CTA;  
518 Yasoshima et al., 2006). Loss of memory has previously been attributed to inhibition of  
519 Fos in central amygdala (Lamprecht et al., 1996), or in the amygdala as a whole along  
520 with the GC (Yasoshima et al., 2006). Our demonstration that knockout restricted to  
521 BLApn is sufficient to impair memory does not contradict these earlier studies, but  
522 suggests that these projection neurons may be a nexus or bottleneck vital for learning in  
523 the circuit.

524 There is still much to be learned about the specific involvement of new transcription of  
525 Stk11 in CTA. This transcript is presumably less transient than that of immediate early  
526 genes, and may be part of a process with complex dynamics. This issue is brought into  
527 focus by the fact that, across the time points measured, the transcript in profiled cells was  
528 decreased, while in anatomically sub-dissected portions of BLA, Stk11 protein was  
529 increased. Improved temporal and spatial mapping of transcript and protein levels will  
530 clarify the nature of the process. Regardless, however, loss of function confirms the  
531 necessity of Stk11 expression within BLApn for CTA learning.

### 532 **Necessity of Stk11 implicates the AMP-related kinase pathway in learning**

533 Prior studies of CTA and other forms of aversive learning in the BLA have implicated a  
534 number of kinases: including those in the cAMP-dependent protein kinase, protein kinase  
535 C, extracellular signal-regulated, and mitogen-activated protein kinase pathways  
536 (Johansen et al., 2011; Adaikkan and Rosenblum, 2012). Each of these also have well

537 established roles in other forms of forebrain learning and plasticity (Alberini, 2009). Here  
538 we reveal the likely involvement of another kinase cascade, well studied in the contexts  
539 of cell growth, metabolism, cancer and polarity (Shackelford and Shaw. 2009) but hitherto  
540 unstudied in the context of learning and memory. That this pathway should have a role in  
541 learning is perhaps not shocking given the ubiquity of its previously demonstrated roles  
542 in 1) axonal development (Barnes et al., 2007; Shelly et al., 2007); 2) synaptic remodeling  
543 during aging (Samuel et al., 2014); 3) regulation of presynaptic neurotransmission (Kwon  
544 et al. 2016); and perhaps most tellingly 4) regulation of glucose metabolism, feeding and  
545 obesity through actions in multiple tissues including hypothalamus (Xi et al., 2018; Fei-  
546 Wang et al., 2012; Claret et al., 2011). Given the involvement of hypothalamus in coding  
547 of taste palatability, and the connectivity between hypothalamus and gustatory cortex (Li  
548 et al., 2013), it is tempting to speculate that the role of Stk11 signaling pathways in feeding  
549 may be functionally related to its role in gustatory learning. Further studies will be needed  
550 to distinguish whether the involvement of Stk11 in memory is specific to forms of learning  
551 regulating consumption, and whether its role in CTA learning is confined to the basolateral  
552 amygdala.

553 Stk11 is a master kinase that regulates the activity of 13 downstream AMP-related  
554 kinases with diverse roles (Lizcano et al., 2004). Prkaa1/2 (also known as AMPK) is  
555 crucial for metabolic regulation during altered levels of nutrients and intracellular energy.  
556 BRSK and MARK regulate cell polarity during development (Barnes et al., 2007;  
557 Shackelford and Shaw. 2009). We found that, while Prkaa1/2 and several other  
558 downstream kinases have low levels of expression in BLA<sup>n</sup>, others, including Mark2 and  
559 3, are expressed at higher levels (Figure 2-figure supplement 3). Furthermore, changes  
560 in MARK2 and Stk11 expression were correlated during CTA learning. During axonal  
561 development, BDNF and cAMP signaling require the Stk11/MARK cascade (Barnes et  
562 al., 2007; Shelly et al., 2007). BDNF and cAMP are also implicated in CTA (Ma et al.,  
563 2011; Koh et al., 2002; Koh et al., 2003), raising the possibility that these signaling  
564 pathways also intersect during learning.

565 Deletion of Stk11 prior to training profoundly impaired memory, but deletion two days after  
566 training—when memory formation and consolidation have already occurred (Alberini,  
567 2009; Gal-Ben-Ari et al., 2012; Bambha-Mukku et al., 2014; Levitan et al., 2016) —did  
568 not. This suggests that Stk11 expression in BLA<sup>n</sup> promotes memory formation, rather  
569 than memory maintenance or retrieval. It is clear that Stk11 deletion left much of the  
570 machinery of taste processing and learning intact, however. Activation of Fos by training  
571 in the BLA was not impaired after Stk11 deletion, implying that at least the initial stages  
572 of transcriptional activation associated with learning are intact. Also left intact was the  
573 ability of the BLA to convey palatability information to the GC. Prior studies have shown  
574 that silencing of BLA neurons, or of their axons within the GC, impair palatability coding  
575 during the late phase of GC gustatory responses. We found that these responses were  
576 still present in GC following knockout, implying that this critical function of the BLA for  
577 CTA learning remained intact.

578

579 **Intrinsic excitability of BLApn as a candidate mechanism for Fos and Stk11's**  
580 **effects on CTA memory.**

581 Learning paradigms that support synaptic plasticity also frequently induce changes in  
582 neuronal excitability, and such plasticity of intrinsic excitability has long been known to  
583 accompany classical conditioning in the neocortex, olfactory cortex, hippocampus,  
584 amygdala and cerebellum (for reviews see Zhang and Linden, 2003; Frick and Johnston,  
585 2005; Mozzachiodi and Byrne, 2010; Titley et al. 2017; Debanne et al. 2019).

586 In our hands, two genetic manipulations of BLApn that impair learning also increase the  
587 intrinsic excitability of BLApn, whereas BLAp involved in normal conditioning appear to  
588 experience the opposite change. This suggests an important role in learning for  
589 excitability changes, and begs the question of mechanism. Although there are likely  
590 multiple such mechanisms, the increase in excitability partly reflects an increase in the  
591 resting input resistance and a corresponding decrease in the threshold current needed to  
592 evoke firing. It is worth noting that most prior studies of intrinsic plasticity have reported  
593 increases in excitability with learning (Zhang and Linden, 2003; Mozzachiodi and Byrne,  
594 2010; Pignatelli et al., 2019). Our results are not without precedent, however; and are  
595 similar in polarity to those found in BLApn following olfactory fear conditioning, another  
596 form of negative reinforcement learning (Motanis et al., 2014). Decreased excitability of  
597 Fos-activated neurons was also found using an earlier reporter of Fos promoter activation  
598 (Yassin et al., 2010).

599 In conclusion, we have demonstrated dual roles for the kinase Stk11 in BLApn.  
600 Conditional deletion increases their neuronal excitability and at the same time blocks  
601 acquisition of CTA memory without altering baseline contributions to taste coding or the  
602 ability to undergo the initial stages of transcriptional activation during training. Further  
603 work will be needed, first, to map out the intervening steps by which Stk11 affects  
604 downstream signaling partners leading to increased excitability and reduced learning,  
605 second, to better understand how these pathways intersect with transcriptional activation  
606 of immediate early genes, and third, to determine whether, and if so how, cellular changes  
607 in excitability and behavioral changes in learning are causally related.

608

609 **Material and Methods**

610 **Subjects**

611 Male and Female mice were used for behavior at age 60-80 days, or for electrophysiology  
612 at 25-35 days. Strains: wild-type; WT (C57BL/6J), YFP-H (B6.Cg-Tg(Thy1-YFP)HJrs/J),  
613 Stk11<sup>fl/fl</sup> (B6(Cg)-Stk11tm1.1Sjm/J, Lkb1fl), Fos<sup>fl/fl</sup> (B6;129-Fostm1Mxu/Mmjx), Fos-tTA  
614 (B6.Cg-Tg(Fos-tTA,Fos-EGFP\*)1Mmay/J) all purchased from Jackson Laboratories (Bar  
615 Harbor, ME, USA). Mice were placed on a 12-hour light-dark cycle, and given *ad libitum*  
616 access to food and water except during training, at which time water access was

617 restricted, while food remained available *ad libitum* (note that animals reliably consume  
618 less food when thirsty). All procedures were approved by the Brandeis University  
619 Institutional Animal Care and Use Committee (IACUC) in accordance with NIH guidelines.

620 **Surgery**

621 **BLA cannulation for RNA synthesis inhibition experiments:** WT Mice were  
622 anesthetized via ip injections of 100 $\mu$ g ketamine, 12.5  $\mu$ g xylazine, 2.5  $\mu$ g acepromazine  
623 per gram (KXA). Guide cannulae (23-gauge, 10 mm length) were implanted above the  
624 BLA (mm from bregma, AP= -1.4, DV= 4.2, ML=  $\pm$ 3.4) and stabilized using Vetbond and  
625 dental acrylic. Stainless steel stylets (30-gage, 10mm) were inserted into the guide  
626 cannula to ensure patency. Mice received postsurgical metacam (5  $\mu$ g/g), penicillin (1500  
627 Units/g) and saline (5 % body-weight) per day for three days and recovered a total of 7  
628 days prior to training. Twenty minutes prior to CTA training, mice were infused with either  
629 50 ng of actinomycin-D or vehicle control (PBS) bilaterally (in 1  $\mu$ l over 2 minutes) via  
630 infusion cannulae extending 0.5 mm below the guide cannulae to reach the BLA. Each  
631 cannula was connected to a 10 $\mu$ l Hamilton syringe on a syringe pump (Harvard  
632 Apparatus, Massachusetts, MA, USA).

633 **BLA viral infection:** Stk11<sup>ff</sup>, Fos<sup>ff</sup> and Fos-tTA mice were anesthetized with KXA. The  
634 skull was exposed, cleaned, and bilateral craniotomies were made at stereotactic  
635 coordinates (AP= -1.4, ML=  $\pm$ 3.4). BLA were injected bilaterally with AAV2/5-  
636 Camk2 $\alpha$ ::Cre-GFP or AAV2/5-Camk2 $\alpha$ ::GFP (UNC, vector core) for Stk11<sup>ff</sup> and Fos<sup>ff</sup>  
637 mice and AAV2/5-TRE::mCherry for Fos-tTa mice, 10 days prior to CTA training using  
638 sterile glass micropipettes (10-20  $\mu$ m diameter) attached to a partially automated  
639 microinjection device (Nanoject III Microinjector, Drummond Scientific). The  
640 micropipettes were lowered to 4.3 mm and 4.6 mm from the dura to reach the BLA. At  
641 each depth, virus (200 nl) was delivered via 10 pulses of 20 delivered every 10 sec, with  
642 10 min between each injection. Postsurgical treatment and recovery were as above.

643 **Conditioned Taste Aversion (CTA):** Mice were housed individually with free access to  
644 food and maintained on a 23.0 h water deprivation schedule for the duration of training  
645 and experimentation. Three days prior to CTA training, water bottles were removed from  
646 the cages and water was given twice a day (10 am and 6 pm) for a duration of 30 min.  
647 On the day of CTA mice were given 30 min to consume 0.5 % saccharin, which was  
648 followed by intraperitoneal (I.P) injection of lithium-chloride (0.15M, 2% of body weight,  
649 unless indicated differently) 30 min later. Taste control groups received I.P injection of  
650 saline (0.9 % sodium chloride) instead of lithium and lithium control group received lithium  
651 injection alone 24 hours following Saccharin consumption. CTA testing: Mice were kept  
652 on watering schedule twice a day and 48 hours after CTA training mice received CTA  
653 testing which consisted of 30 min consumption of 0.5 % saccharin. 8 hours following  
654 testing mice were given 30 min water consumption.

655 **Seizure induction:** Fos<sup>ff</sup> mice were housed individually with free access to water and  
656 food and received BLA viral infection with Cre and control viruses as described above.

657 After 10 days, mice were injected I.P. with 20 mg/kg kainic acid in PBS. Four hours after  
658 injection, mice were perfused for Fos immunohistochemistry.

659 **Immunohistochemistry:** Mice were deeply anesthetized with an overdose of KXA and  
660 perfused transcardially with phosphate buffered solution (PBS) followed by 4%  
661 paraformaldehyde (PFA). Brains were post-fixed in PFA for 1-2 day, and coronal brain  
662 slices (60  $\mu$ m) containing the BLA (-1mm to -2.5 mm anterior-posterior axis) were  
663 sectioned on a vibratome. Slices were rinsed with PBS and incubated in a blocking  
664 solution (PBS/.3%TritonX-100/5% Bovine serum albumin) for 12-24 hours at 4°C.  
665 Blocking solution was removed and replaced with the primary antibody solution which  
666 consists of 1:100 c-Fos polyclonal rabbit IgG (SC-52G; Santa Cruz Biotechnology) for 24  
667 hours at 4°C. After incubation, slices were rinsed using a PBS/.3% Triton X-100 solution  
668 followed by the secondary antibody incubation of 1:500 c-Fos Alexa Flour 546 Goat-Anti-  
669 Rabbit IgG (H+L) (Life Technologies) and 5% natural goat serum for 12-24 hours at 4°C.  
670 Sections were then rinsed 5-6 times over 90 mins (1XPBS/.3% Triton X-100),  
671 counterstained with DAPI, mounted with antifade mounting medium (Vectashield), and  
672 viewed by confocal fluorescence microscopy (Leica Sp5 Spectral confocal  
673 microscope/Resonant Scanner). Imaging and quantification were performed blind to  
674 experimental group.

675 **Fos quantification and analysis:** To minimize systematic bias, Fos counts were  
676 performed blind and semi-automatically, using Fiji (University of Wisconsin-Madison;  
677 Schindelin et al. 2012). Eight-bit images were binarized and particles smaller than 10  
678  $\mu$ m<sup>2</sup> were rejected. Each cell count was from a separate animal and was the average of  
679 counts from six sections through the anterior, middle and posterior regions of the BLA of  
680 both hemispheres.

681 **RNA sequencing experiment:** RNA sequencing was performed on YFP<sup>+</sup> BLApn  
682 harvested from male YFP-H mouse line (Feng et al., 2000; Sugino et al., 2006; Jasnow  
683 et al., 2013; McCullough et al., 2016) which expresses YFP under the Thy1 promoter in  
684 the majority of excitatory projection neurons located in the anterior part of the nucleus.  
685 The mice underwent CTA training or taste-only controls (n=4/group) and 4 hours following  
686 training were subjected to manual cell-sorting, performed as previously described (Sugino  
687 et al, 2006; Hempel et al; 2007; Shima et al., 2016) by dissociating 150-200 fluorescently  
688 labeled neurons in 300 $\mu$ m thick brain slices and manually purifying them through multiple  
689 transfer dishes with the aid of a pipette viewed under a fluorescence dissection  
690 microscope. Total RNA was extracted from sorted cells using Pico-pure RNA isolation kit  
691 (Thermo fisher). Amplified cDNA libraries are prepared from isolated, fragmented RNA  
692 using the NuGen Ovation RNAseq V.2 kit (NuGEN, San Carlos, CA) and followed by  
693 purification using the Beckman coulter Genomic's Agencourt RNA Clean XP kit and Zymo  
694 DNA Clean & Concentrator. Sequencing adaptors are ligated per Illumina protocols and  
695 50 bp single-ended reads are obtained from Illumina Hi-Seq machine. Libraries  
696 sequenced usually results in 25-30 million unique reads using 8-fold multiplexing.

697 **Analysis:** Reads are trimmed and then aligned to the mouse genome using TopHat  
698 (Trapnell et al. 2012). Sam files are converted to binary format using Samtools and  
699 visualized at the sequence level using IGV. Script written in python and R statistical  
700 package are used to convert unique reads to gene expression values and to filter genes  
701 by relative expression and statistical significance.

702 **qPCR validation:** The brains of a separate group of YFP-H mice receiving CTA  
703 (taste+lithium, N=4) or taste control (taste+saline, N=4) were harvested 4 hours following  
704 the end of the training and subjected to fax sorting. RNA was extracted using pico-pure  
705 kit and reverse transcribed to cDNA using iScript cDNA synthesis kit. qPCR was  
706 performed on Rotor-Gene qPCR machine using PCR master mix and transcript-specific  
707 sets of primers for Fos, Stk11 as target genes and Snap47 as loading control.

708 **Acute slice electrophysiology:** Ten days after virus injection, acute brain slices were  
709 prepared from P28-35 mice. Animals were deeply anesthetized with KXA and  
710 transcardially perfused with ice-cold oxygenated cutting solution containing (in mM): 10  
711 N-methyl-D-glucamine (NMDG), 3 KCl, 1 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 20 HEPES, 2  
712 Thiourea, 3 Sodium Pyruvate, 12 N-acetyl-L-cysteine, 6 MgCl<sub>2</sub>, 0.5 CaCl<sub>2</sub>, 5 Sodium  
713 Ascorbate, 10 Glucose (pH: 7.25 – 7.4, adjusted using HCl). 300  $\mu$ m coronal slices  
714 containing the BLA were cut on a vibratome (Leica), and then recovered for 15 min at 33  
715 °C and for 15 min at room temperature in oxygenated recovery solution containing (in  
716 mM): 74 NaCl, 3 KCl, 1 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 6 MgCl<sub>2</sub>, 0.5 CaCl<sub>2</sub>, 5 Sodium  
717 Ascorbate, 75 Sucrose, 10 Glucose, followed by at least another 1 hour at room  
718 temperature in oxygenated ACSF containing (in mM): 126 NaCl, 3 KCl, 1 NaH<sub>2</sub>PO<sub>4</sub>, 25  
719 NaHCO<sub>3</sub>, 2 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 10 Glucose. During recordings, slices were perfused with  
720 oxygenated 34-35 °C ACSF. Target neurons in BLA were identified based on the  
721 presence of viral GFP reporter. ACSF included 35  $\mu$ M d,l-2-amino-5-phosphonovaleric  
722 acid (APV) and 20  $\mu$ M 6,7-dinitroquinoxaline-2,3-dione (DNQX) to block ionotropic  
723 glutamate receptors, and 50  $\mu$ M picrotoxin to block ionotropic GABA receptors. Whole-  
724 cell recording pipettes (6 – 8 M $\Omega$ ) were filled with internal solution containing (in mM): 100  
725 K-gluconate, 20 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, and  
726 0.1% biocytin. Recordings were amplified (Multiclamp 700B, Molecular Devices) and  
727 digitized at 10 kHz using a National Instruments Board under control of IGOR Pro  
728 (WaveMetrics). Resting membrane potentials were adjusted to -70 mV and steady state  
729 series resistance was compensated. Series resistance and input resistance were  
730 calculated using -5 mV (voltage clamp) or 25 pA (current clamp) seal tests before each  
731 trial of recording. Measurements of input resistance in Fos reporter labeled neurons  
732 (Figure 7) were measured in voltage clamp, all other recordings were performed in current  
733 clamp. The calculated liquid junction potential (-10 mV) was compensated post hoc.  
734 Neurons with high series resistance (> 30 M $\Omega$  current clamp) or membrane potentials that  
735 changed by > 10 mV were excluded. Hyperpolarization activated sag was measured from  
736 responses to -100 pA current steps. Action potential (AP) threshold, amplitude,  
737 afterhyperpolarization (AHP) and full width at half-height were averaged from the 5th-10th  
738 APs in trials with 10 to 20 Hz firing rates. AP threshold is the membrane potential at which

739 the slope first exceeds 10 V/s, and AP amplitude was measured relative to threshold. Sag  
740 ratio is defined as the fraction by which the membrane potential depolarized at steady-  
741 state from its maximum hyperpolarization during a -100 pA current step. Medium AHP  
742 was measured as the peak hyperpolarization after the APs mentioned above relative to  
743 threshold. The slow AHP was measured from the peak hyperpolarization following  
744 positive current steps generating 10-20 Hz firing.

745 ***In-vivo recording of GC taste responses***

746 ***Surgery:*** *Stk11<sup>fl/fl</sup>* mice were anesthetized and prepared for stereotaxic surgery and viral  
747 infection as above. Each mouse was also implanted bilaterally with multi-channel  
748 electrode bundles (16 formvar-coated, 25- $\mu$ m diameter nichrome wires) in GC (Distance  
749 from Bregma: AP=+1.2mm; ML=±3 mm; DV of -2.25 mm from the *pia mater*) and a single  
750 intraoral cannula (IOC; flexible plastic tubing) was inserted into the cheek to allow  
751 controlled delivery of taste stimuli. 24 hours before recording sessions began electrode  
752 bundles were then further lowered by 0.75-1.00 mm to reach ventral GC (see Figure 5A).

753 ***IOC Fluid delivery protocol:*** Experiments began with three days of habituation to the  
754 recording setup and to receiving liquid through the IOC. Sixty 15- $\mu$ l aliquots (hereafter,  
755 “trials”) of water were delivered across 30 min. To ensure adequate hydration, mice were  
756 given two 30 min period of access to additional water.

757 On the following day, recording commenced and water trials were replaced with 4  
758 different taste stimuli: sweet (0.2 M sucrose), salty (0.1 M sodium chloride), sour (0.02 M  
759 citric acid), and bitter (0.001 M quinine). A total of 15 trials were delivered for each taste  
760 in random order. These tastes and concentrations were chosen because they provided a  
761 broad range of hedonic values for palatability assessment. Fluid delivery through a  
762 nitrogen-pressurized system of polyethylene tubes was controlled by solenoid valves via  
763 a Raspberry Pi computer (construction details and code available on request from  
764 [[https://github.com/narendramukherjee/blech\\_clust](https://github.com/narendramukherjee/blech_clust)]).

765 ***Taste palatability coding:*** To determine whether a neuron displays palatability activity,  
766 we performed a moving window analysis (window size: 250 ms; step size: 25 ms) to trace  
767 the dynamics of taste processing in GC. For each time window, we calculated a  
768 Spearman product-moment correlation between the ranked firing rates to each taste and  
769 the palatability rankings obtained previously in separate experiments (Levitin et al.,  
770 2019).

771 ***Statistical analysis:*** The results are expressed as means ± s.e.m unless otherwise  
772 stated. All effects were evaluated using either paired t-test or one- or two-way ANOVA  
773 test with post hoc t-tests corrected (Bonferroni) for all possible pair-wise comparisons.

774

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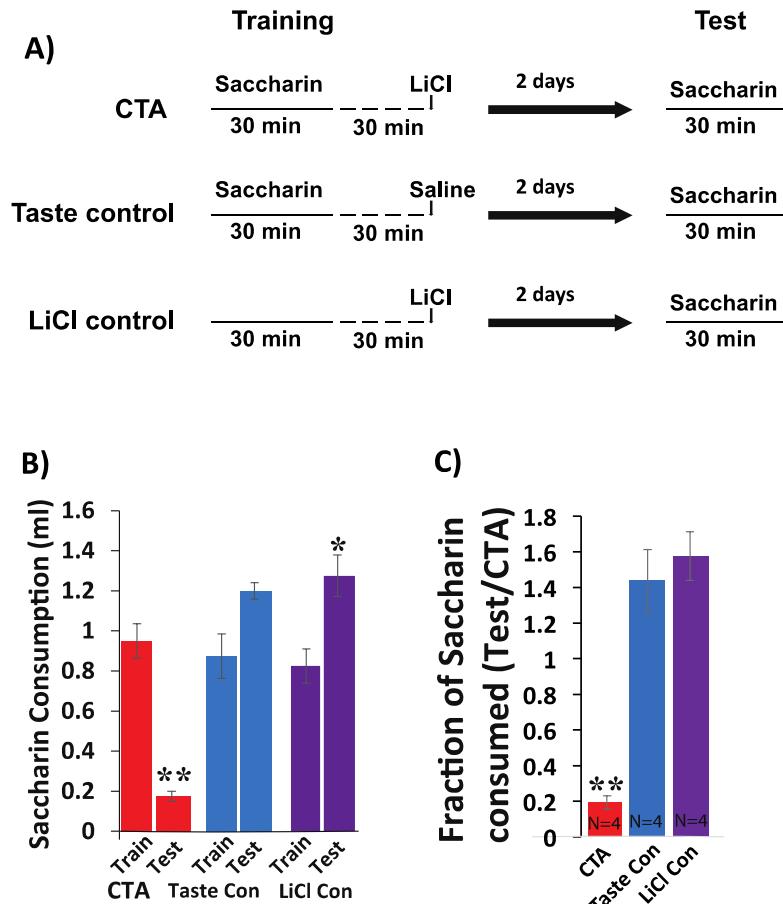
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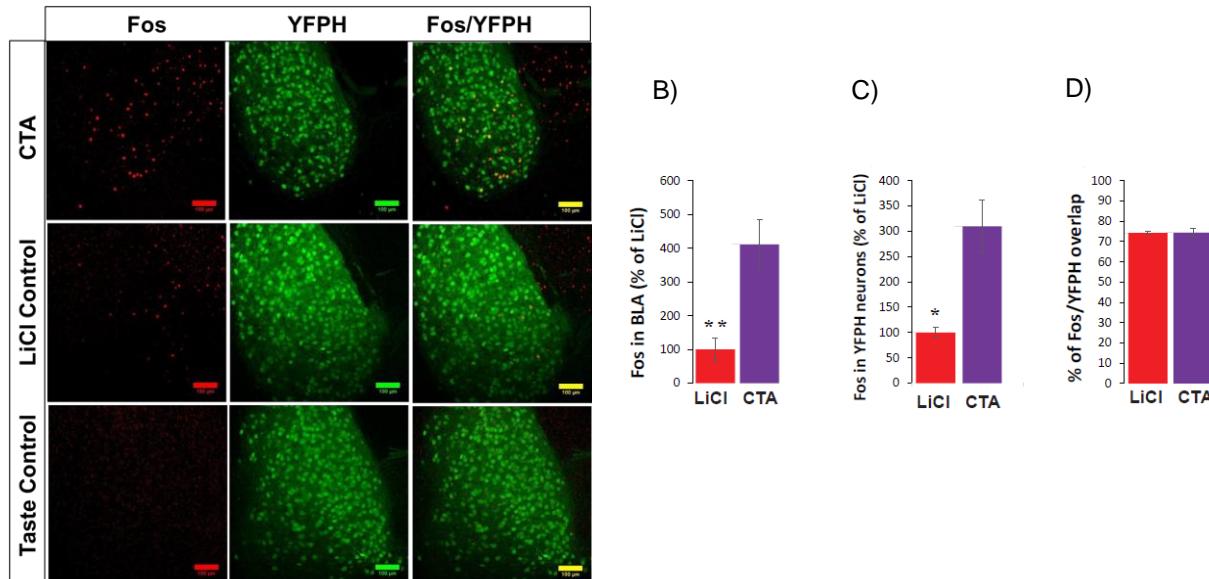
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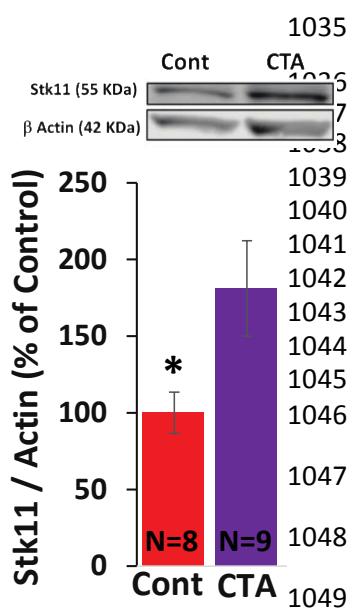
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1006 **Figure 1-figure supplement 1. Testing CTA in mice.** (A) Time line showing behavioral  
1007 paradigm. CTA and Taste control receive 30 min of 0.5% saccharin consumption followed by  
1008 intraperitoneal injection (I.P) injection of lithium-chloride (LiCl; 0.15 M, 2% body-weigh) or saline  
1009 30 min later. The lithium control group receives the same I.P injection of LiCl, but no saline during  
1010 the training phase. All groups were tested for consumption of saccharin during 30 min exposure  
1011 48 hours later. (B) Saccharin consumption decreases following LiCl-induced malaise  
1012 (N=4/group). There was main effect on drinking volume of saccharin between the groups across  
1013 sessions:  $f(4,18)=28.63$ ,  $p=1E-7$  A subsequent pairwise comparison between training and test  
1014 revealed  $p=4E-5$  for CTA group,  $p=0.016$  for LiCl group and  $p=0.172$  for taste group. (C)  
1015 Measuring CTA memory strength. The strength of CTA memory was determined by quantifying  
1016 the fraction of saccharin consumption in the test day out of the consumption in the training day.  
1017 Comparing CTA strength of all groups revealed main effect for difference between groups  
1018  $f(2,9)=34.08$ ,  $p=6E-5$ ;  $p=2.5E-4$  for CTA vs taste control and  $p=0.00011$  for CTA vs LiCl control  
1019 and  $p=1$  for taste control vs LiCl control (On way ANOVA with Bonferroni correction). \* $p<0.05$ ;  
1020 \*\* $p<0.01$ .labeled neurons from the BLA in the YFP-H mouse line (Feng et al., 2000; Sugino et al.,  
1021 2006; Jasnow et al., 2013; McCullough et al., 2016) which expresses YFP under the Thy1  
1022 promoter in a large population of excitatory projection neurons located in the anterior part of the  
1023 nucleus.



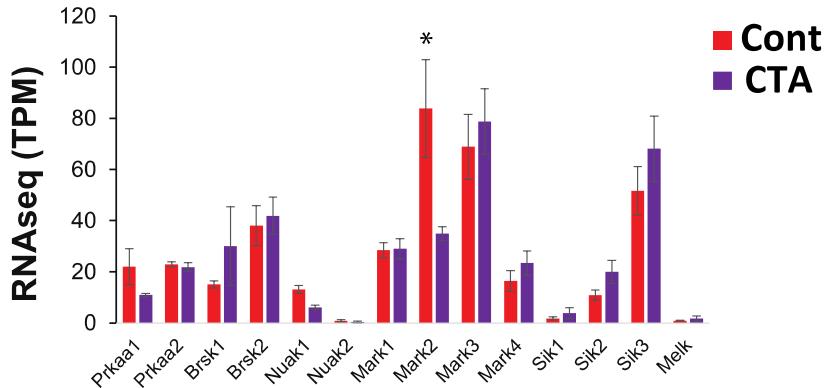
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1025 **Figure 2-figure supplement 1. CTA increases Fos protein expression in BLApcn including**  
1026 **those in strain YFP-H.** (A) Images of YFP<sup>+</sup> neurons in the BLA (green) and Fos protein (red) 4  
1027 hours following CTA training, LiCl and taste controls. Note that the taste control group has no  
1028 Fos<sup>+</sup> signal and so is not shown in B-D. (B) CTA increased Fos expression in BLA relative to the  
1029 LiCl and taste controls ( $F(2,11)=21.2$ ;  $p=4E-4$ ; Post hoc (Bonferroni corrected) difference between  
1030 CTA and LiCl groups:  $p=0.003$ , and  $p=4E-4$  between CTA and taste control groups,  $N=4$ /group).  
1031 (C) As in (B), but only for Fos overlapping YFP expression ( $F(2,11)=23.5$ ;  $p=0.008$ , post hoc: CTA  
1032 and taste control groups:  $p=4E-4$ ;  $N=3-4$ /group). \* $p<0.05$ ; \*\* $p<0.01$ . (D) In both CTA and LiCl  
1033 control conditions the expression of Fos protein was similarly localized to YFP<sup>+</sup> neurons (74.4%  
1034 for CTA and 74.2% for LiCl control).



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1036 **Figure 2-figure supplement 2.** Stk11 protein expression  
1037 following CTA training. YFP-H mice were trained for CTA or  
1038 received a taste control and 4 hours later the anterior BLA  
1039 (guided by YFP expression) was subdissected and used for  
1040 immunoblotting with antibodies raised against Stk11 and actin  
1041 (as loading control). CTA increased the expression of Stk11  
1042 ( $t(15)=2.28$ ;  $p=0.037$ ,  $N=8/9$  per group). \* $p<0.05$ .

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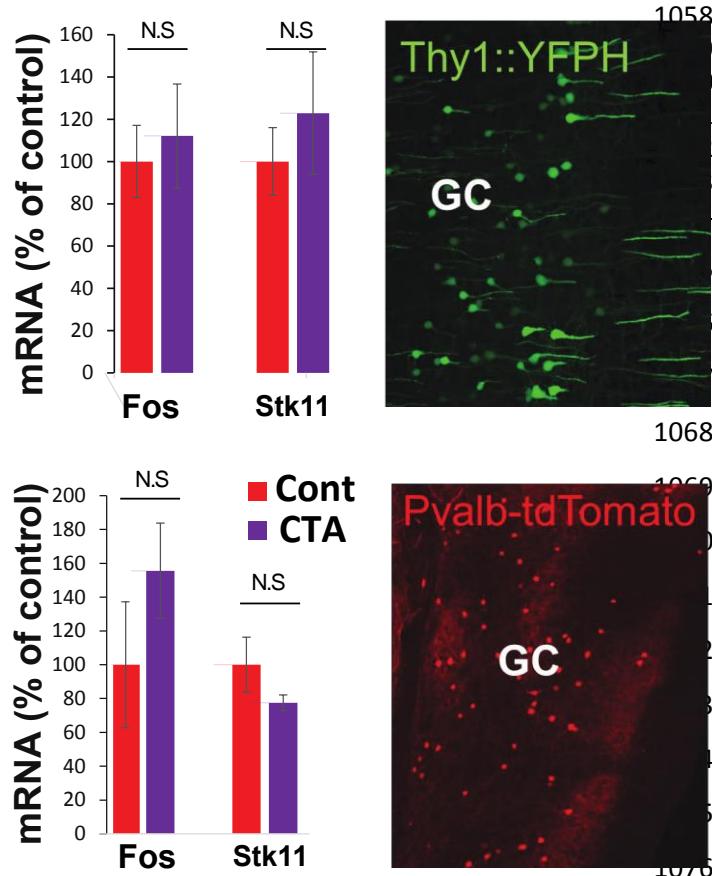
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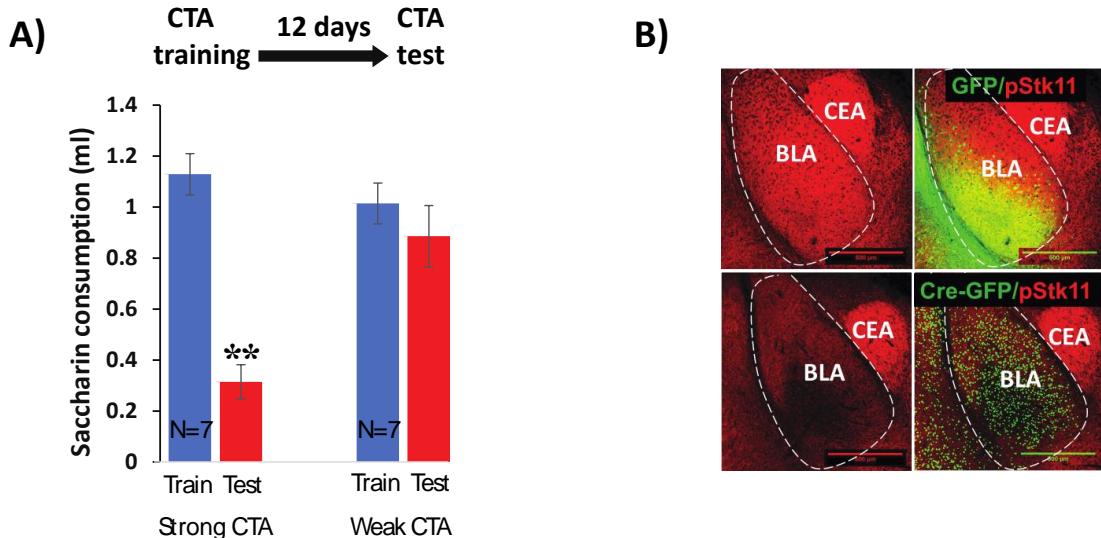
1053 **Figure 2-figure supplement 3. BLApc transcript levels of known downstream substrates of Stk11,**  
1054 **the members of the AMP-related kinase family (Lizcano et al., 2004).** RNA sequencing from BLApc 4  
1055 hours following CTA training. Note that Mark2 mRNA expression is reduced in taste control mice relative  
1056 to CTA trained mice (N=4/group; \*p<0.05); TPM- transcripts per million.

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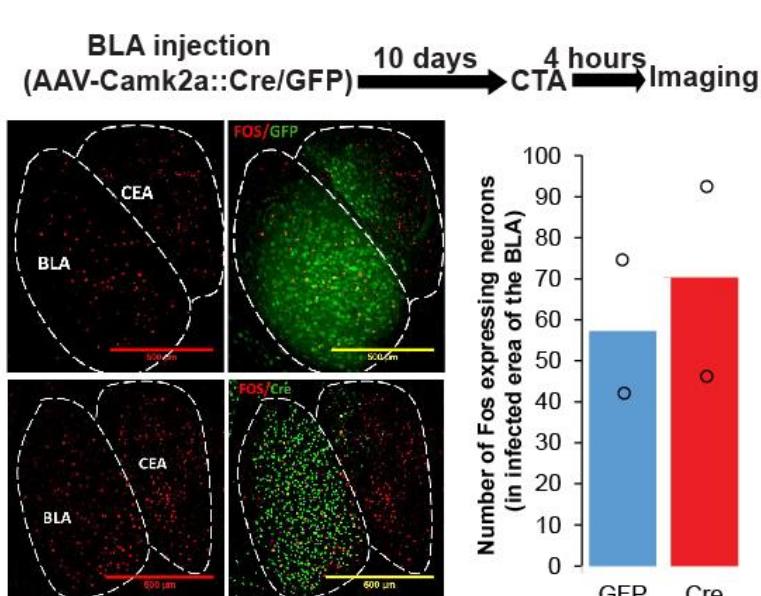


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Figure 2-figure supplement 4. RNA sequencing from GC. Top. YFP-H L5 pyramidal neurons and Pvalb-tdTomato positive interneurons in the GC. Fos and Stk11 did not differ significantly between CTA and taste control groups.



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1078 **Figure 4-figure supplement 1.** (A) CTA elicited by a dose of 0.15 M LiCl 1% body weight (Weak CTA)  
1079 does not last 12 days following training (N=7,  $t(12)=0.89$ ,  $p=0.391$ ). On the other hand CTA elicited by a  
1080 LiCl dose of 0.15M 2% body weight (Strong CTA) does (N=7,  $t(12)=7.757$ ;  $p=5.15E-06$ ). (B) Conditional  
1081 knock-out of Stk11 reduces phospho-Stk11 protein expression. Immunostaining for Stk11 protein  
1082 (phosphorylated at serine 431) was performed 10 days after BLA infection with Cre or control viruses. Scale  
1083 bar: 500  $\mu$ m.



1084  
1085 **Figure 5- figure supplement 1.**  
1086 **CTA induced Fos protein**  
1087 **expression persists after Stk11**  
1088 **deletion in BLA**. Fos protein  
1089 expression in the BLA was  
1090 measured 4 hours following CTA  
1091 training in Stk11<sup>ff</sup> receiving Cre or  
1092 control virus injection 10 days  
1093 earlier.

1094 **Table 1. Transcripts in YFP<sup>+</sup> BLApn with significantly altered expression 4 hours**  
 1095 **following CTA.** Criteria:  $2 \leq \text{fold change} \leq 0.5$ ,  $p < 0.01$ ,  $\text{TPM} > 30$  ( $\text{TPM} = \text{transcript per}$   
 1096  $\text{million}$ ).

Symbol	Fold-Change	P-Value	Gene name
<b>Upregulated in CTA vs taste control</b>			
Nptx1	2.02	1.57E-4	Neuronal pentraxin 1
Ric8	2.06	0.0017	RIC8 guanine nucleotide exchange factor A
Mmab	3.66	0.0019	methylmalonic aciduria (cobalamin deficiency) cblB type homolog
1110008F13Rik	2.08	0.0028	RAB5 interacting factor
Kbtbd4	2.09	0.0044	kelch repeat and BTB (POZ) domain containing 4(Kbtbd4)
Nudt21	2.14	0.0077	nudix (nucleoside diphosphate linked moiety X)-type motif 21
Fos	2.64	0.0094	FBJ osteosarcoma oncogene
Magoh	2.72	0.0095	mago homolog, exon junction complex core component
<b>Downregulated in CTA vs taste control</b>			
Surf2	0.40	5.42E-4	surfeit gene 2(Surf2)
Tmem136	0.39	8.69E-4	transmembrane protein 136
Stk11	0.50	0.0011	serine/threonine kinase 11(Stk11)
Kank3	0.28	0.0022	KN motif and ankyrin repeat domains 3
Lrrn1	0.49	0.0024	leucine rich repeat protein 1, neuronal
Trpc1	0.48	0.0032	transient receptor potential cation channel, subfamily C, memb. 1
Prpf6	0.49	0.0045	pre-mRNA splicing factor 6
Tctex1d2	0.49	0.0052	Tctex1 domain containing 2
Gpr108	0.37	0.0056	G protein-coupled receptor 108
Vkorc1	0.39	0.0069	vitamin K epoxide reductase complex, subunit 1
D10Wsu102e	0.50	0.0082	DNA segment, Chr 10, Wayne State University 102, expressed
Tmem107	0.28	0.0089	transmembrane protein 107

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1098 **Table 2. Transcripts in YFP<sup>+</sup> L5 pyramidal neurons in the GC with significantly**  
 1099 **altered expression 4 hours following CTA.** Criteria:  $2 \leq \text{fold change} \leq 0.5$ ,  $p < 0.01$ ,  $\text{TPM}$   
 1100  $> 30$ .

Gene Symbol	Fold-Change	P-Value	Gene name
<b>Transcript Down-regulated CTA vs taste control</b>			
Exosc1	0.43	0.008	exosome component 1

1101

1102 **Table 3. Transcripts in Pvalb<sup>+</sup> interneurons in the GC with significant altered**  
1103 **expression 4 hours following CTA.** Criteria: 2 ≤ fold change ≤ 0.5, p<0.01, TPM > 30.

Symbol	Fold-Change	P-Value	Gene name
<b>Upregulated in CTA vs taste control</b>			
Uprt	3.38	0.001	uracil phosphoribosyltransferase
Snca	2.66	0.001	synuclein, alpha
1810043H04Rik	2.57	0.004	NADH:ubiquinone oxidoreductase complex Assemb.Fact. 8
Dedd	2.47	0.001	death effector domain-containing
Fam149b	2.26	0.007	family with sequence similarity 149, member B
Jazf1	2.12	0.008	JAZF zinc finger 1
Nup54	2.09	0.007	nucleoporin 54
<b>Downregulated in CTA vs taste control</b>			
Dear1	0.006	0.0006	dual endothelin 1/angiotensin II receptor 1
Nt5c3b	0.20	0.002	5'-nucleotidase, cytosolic IIIB
Lrrc16b	0.26	0.010	capping protein regulator and myosin 1 linker 3
Enc1	0.31	0.005	ectodermal-neural cortex 1
Asap2	0.38	0.004	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2
Pacs2	0.38	0.0002	phosphofuran acidic cluster sorting protein 2
Ncan	0.42	0.008	neurocan
uc008jhl.1	0.42	0.010	
Smarcd3	0.45	0.006	SWI/SNF Related, Matrix Assoc.Actin Dep.Reg. Chromatin, Subfamily D, Member 3
Tbce	0.46	0.002	tubulin-specific chaperone E
uc009mzt.1	0.47	0.008	
Anxa6	0.50	0.001	annexin A6

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1113 **Table 4. Electrophysiological properties of BLApn: Stk11 knockout vs. GFP**  
 1114 **controls.**

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Group	Statistics	Resting membrane potential (mV)	Access resistance (mΩ)	Input Resistance (mΩ)	mAHP (mV)	sAHP (mV)	Action potential Amplitude (mV)	Action potential half width (ms)	Action potential threshold (mV)	Sag ratio
KO	mean	-78.23	19.68	105.33	7.44	0.92	74.41	0.80	-50.01	0.13
	S.D.	3.73	2.76	24.88	1.93	0.35	6.58	0.10	2.44	0.04
GFP	mean	-76.96	18.02	85.41	9.71	0.64	78.05	0.79	-48.30	0.15
	S.D.	2.66	3.47	17.65	1.64	0.26	4.39	0.07	2.55	0.03
	F	2.59	4.47	14.41	26.69	13.78	7.17	0.19	7.68	7.48
	p	0.112	0.0383	3.29E-4	2.54E-06	4.32E-4	0.0094	0.664	0.0073	0.008

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1119 **Table 5. Electrophysiological properties of BLApn: Fos knockout vs. GFP controls.**

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Group	Statistics	Resting membrane potential (mV)	Access resistance (mΩ)	Input Resistance (mΩ)	mAHP (mV)	sAHP (mV)	Action potential Amplitude (mV)	Action potential half width (ms)	Action potential threshold (mV)
KO	mean	-75.43	19.63	113.91	10.34	0.40	65.87	0.72	-40.79
	S.D.	5.08	4.95	27.73	4.30	0.19	8.31	0.11	5.83
GFP	mean	-76.50	14.35	86.01	8.95	0.38	73.30	0.74	-45.62
	S.D.	3.38	5.17	19.58	2.05	0.17	5.11	0.12	3.02
	F	0.768	13.882	16.812	2.096	0.144	14.334	0.224	13.306
	p	0.385	5.04E-04	1.55E-04	0.154	0.706	4.18E-04	0.638	6.40E-04

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