Supplementary Information Guide

Intercalated amyodala clusters orchestrate a switch in fear state

- 4 Kenta M. Hagihara, Olena Bukalo, Martin Zeller, Ayla Aksoy-Aksel, Nikolaos Karalis, Aaron Limoges,
- 5 Tanner Rigg, Tiffany Campbell, Adriana Mendez, Chase Weinholtz, Mathias Mahn, Larry S. Zweifel,
 - Richard D. Palmiter, Ingrid Ehrlich, Andreas Lüthi, Andrew Holmes

Supplementary Table 1 | Cross-study comparison of ITC cluster nomenclature.

Because different nomenclature and abbreviations were used for individual ITC clusters in previous work, here we organize them from representative references and summarize their correspondence to our definition used in this study. Of note, our detailed reconstructions led us to distinguish ITC_{vm} from a more rostrally located ventral cluster, which we label ITC anteroventral (ITC_{av}). Also, we report a new cluster – ITC posteroventral (ITC_{pv}), which has not been reported previously.

Supplementary Table 2 | Statistical analyses summary for chemogenetic experiments.

For each chemogenetic manipulation group mice with combined unilateral and bilateral, unilateral only and bilateral expression were compared using two-way ANOVA. Separate statistical analyses were conducted for each behavioral stage (fear conditioning, extinction, and retrieval). The F statistics for effects of each variable and interaction of variables are listed and sample size (n) for each group is provided. For retrieval data mean \pm SEM values are shown for Cre- and Cre+ mice. Post-hoc comparisons carried out using Sidak test are indicated as appropriate, separately for baseline and CS during retrieval. Statistical analyses were performed with an α level of 0.05. P values <0.05 were considered significant.

Supplementary Video 1 | 3D-reconstruction of individual ITC clusters.

An example 3D-reconstruction of ITC clusters from a cleared FoxP2-Cre crossed with tdTomato reporter mouse brain. The same sample as shown in **Extended Data Fig.1b** is presented. Note, this intercross mouse line shows tdTomato signal in some CeA neurons and blood vessels in addition to ITCs, likely because of developmental transient expression of FoxP2.

Supplementary Video 2 | Post-hoc identification of ITCdm in a 3D confocal image of a cleared tissue.

An example confocal 3D image stack of cleared and stained tissue acquired with a 20x objective. The same sample as shown in **Fig.1d,e** is presented.

Supplementary Video 3 | Spatially clustered robust aversive shock responses in ITCdm.

Example Ca2+ movies from simultaneously recorded BLA, CeA, and ITCdm regions. ITCdm shows spatially clustered and robust responses to 5 trials of aversive footshock presentation. The video was sped up 2x. The timing of shock presentation is indicated with red 'shock' text. The same sample as shown in **Fig.1f,g** is presented.