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NMDA-receptor-dependent plasticity in the bed nucleus of the stria terminalis triggers long-term anxiolysis

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Anxiety is controlled by multiple neuronal circuits that share robust and reciprocal connections with the bed nucleus of the stria terminalis (BNST), a key structure controlling negative emotional states. However, it remains unknown how the BNST integrates diverse inputs to modulate anxiety. In this study, we evaluated the contribution of infralimbic cortex (ILCx) and ventral subiculum/CA1 (vSUB/CA1) inputs in regulating BNST activity at the single-cell level. Using trans-synaptic tracing from single-electroporated neurons and \textit{in vivo} recordings, we show that vSUB/CA1 stimulation promotes opposite forms of \textit{in vivo} plasticity at the single-cell level in the anteromedial part of the BNST (amBNST). We find that an NMDA-receptor-dependent homosynaptic long-term potentiation is instrumental for anxiolysis. These findings suggest that the vSUB/CA1-driven LTP in the amBNST is involved in eliciting an appropriate response to anxiogenic context and dysfunction of this compensatory mechanism may underlie pathologic anxiety states.

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Axiety is a physiological negative emotion that triggers a state of alert to possible threat and promotes survival. The bed nucleus of the stria terminalis (BNST) belongs to a neuronal network of interconnected limbic regions and exerts a pivotal role in the expression of anxiety both in humans and in rodent models. Previous studies using electrical, lesioning, pharmacological or optogenetic manipulations targeting directly specific BNST nuclei or cell types have been informative but have not been able to determine precisely how different BNST inputs influence anxiety. It has been shown that the ventral subiculum/CA1 and infralimbic cortex (ILCx) massively projects to the BNST. However, the integrative properties of BNST neurons at the single-cell level have never been explored. One hypothesis is that cortical and hippocampal information is processed at the single-cell level in BNST to trigger anxiolysis.

One of the most likely neural mechanisms underlying persistent anxiety is long-lasting plasticity in the neuronal network. Probably owing to their anatomically convergent and segregated excitatory inputs from the infralimbic Cortex (ILCx) and the vSUB/CA1 and their high content in stress-related neuromodulators, BNST circuits display morphological or synaptic plastic adaptations in response to stress and anxiety. Ex vivo studies in slice have correlated plasticity at glutamate synapses within the BNST with alterations in anxiety levels, but it remains unclear how the BNST integrates diverse inputs to modulate anxiety-related behaviors. Synaptic homeostasis seems to be a crucial process to compensate a long-lasting enhancement in signal transmission and maintain the stability of neuronal activity. Recent computational modeling of synaptic plasticity have shown that the homeostatic processes that control the network stability are supported by the interaction of homosynaptic plasticity with heterosynaptic plasticity. To unravel the integrative properties of BNST neurons at the single-cell level and the mechanism of their plastic changes in response to specific input stimulation, we combined conventional tracing, transsynaptic tracing from single-electroporated neurons, in vivo single-cell recordings, pharmacological and behavioral techniques. Here, we demonstrate: (1) that both vSUB/CA1 and ILCx converge on the same amBNST neurons; (2) that in vivo stimulation of the vSUB/CA1 promotes an N-methyl-D-aspartate or N-methyl-D-aspartate (NMDA)-dependent long term potentiation (LTP) at the vSUB/CA1-amBNST synapses (LTPvSUB/CA1), associated with an NMDA-independent long-term depression (LTD) at the ILCx-amBNST synapses (LTDILCx); and (3) that the induction of in vivo NMDA-R-dependent plasticity in the amBNST triggers long-term changes of anxiety state.

Results

BNST neurons are connected to both vSUB/CA1 and ILCx inputs. It has been shown that the BNST could integrate information from the vSUB/CA1 or the ILCx. However, it was unknown whether these inputs were integrated separately by different BNST neurons or if the same BNST neuron integrates both inputs. To address this question, we first injected a retrograde tracer, the cholera-toxin-B subunit (CTb), into the BNST, and confirmed that the BNST receives strong innervations from the vSUB/CA1 and the ILCx (Supplementary Fig. 1a-c). We then injected two different anterograde tracers, Phaseolus vulgaris-leucoagglutinin (PHAL) and biotinylated-dextran-amine (BDA) in the vSUB/CA1 and in the ILCx, respectively (Fig. 1a,b). We used the Fox3 protein as a neuronal marker in the BNST. Using confocal imaging, we showed strong convergences of ILCx and vSUB/CA1 terminal fibers on the same Fox3-positive amBNST neuron (Fig. 1c). By using in vivo electrophysiology in anesthetized rats, we investigated whether amBNST neurons were controlled by both vSUB/CA1 and ILCx projections at the single-cell level. When we tested the evoked responses of all recorded amBNST neurons to the stimulation of the ILCx and the vSUB/CA1, we observed that 85.2% of the recorded neurons responded...
to the stimulation of one of these inputs (Fig. 1d). We quantified that 70% of these responding neurons responded to both ILCx and vSUB/CA1 stimulation, (Fig. 1e,f), whereas only 17% and 13% responded to either ILCx or vSUB/CA1 stimulation, respectively (Fig. 1e). The basal firing rate of the responding amBNST neurons was 0.7 ± 0.2 Hz. The onset of the evoked responses for the ILCx-amBNST pathway was 6.94 ± 0.59 ms and 9.75 ± 0.77 ms for the vSUB/CA1-amBNST pathway ($P < 0.01$). Durations of evoked excitatory responses did not differ between these two pathways (ILCx-amBNST: 12.06 ± 1.06 ms; vSUB/CA1-amBNST: 12.78 ± 1.16 ms). The intensity of stimulation used to trigger evoked responses in the amBNST was in the same range between the two inputs (0.2–1 mA). To further dissect connectivity of amBNST inputs onto single-amBNST neurons, we performed in vivo single-cell electroporation of amBNST neuron followed by retrograde monosynaptic tracing with pseudotyped rabies virus strategy (Fig. 2a,b). Five days after electroporation, retrogradely infected neurons were detected in the ILCx and in the vSUB/CA1 (Fig. 2b). Unfortunately, starter cells were not detected in the amBNST, most probably because rabies virus infection will eventually induce cytotoxicity in infected neurons. Together, our findings confirm that a large population of amBNST neurons was synaptically controlled, at the single-cell level, by both vSUB/CA1 and ILCx inputs.

**HFS$_{vSUB/CA1}$ promotes in vivo input-specific LTD and LTP.** To unravel the integrative properties of BNST neurons at the single-cell level, we combined in vivo single-cell recordings and pharmacological approaches. Given that vSUB/CA1 neurons fire at hundreds of Hertz in basal condition (Supplementary Fig. 2a–c), we first assessed the impact of a high-frequency stimulation (HFS; Supplementary Fig. 2d,e) of the vSUB/CA1 (HFS$_{vSUB/CA1}$), on the ability of the vSUB/CA1–amBNST and ILCx-amBNST synapses to undergo plasticity (Fig. 3a–d). This HFS$_{vSUB/CA1}$, originally described by Abraham et al., induced an extremely long-lasting (>8 days), robust and stable LTP in the projection from vSUB/CA1 to medial prefrontal cortex (mPFC). Here, we established that HFS$_{vSUB/CA1}$ triggers input-specific neuroplastic changes in the BNST (Fig. 3). After 30–40 min, HFS$_{vSUB/CA1}$, amBNST neurons exhibited a 42.1% increase in the vSUB/CA1-evoked spike probability ($LTP_{vSUB/CA1}$) together with a 47.4% decrease in the ILCx-evoked spike probability ($LTD_{ILCx}$; $F_{(1,15)} = 35.97$, $P < 0.0001$ for the effect of HFS$_{vSUB/CA1}$ protocol × input-stimulated interaction; $F_{(1,15)} = 80.60$, $P < 0.0001$ for the effect of the input stimulated, Fig. 3c–e). No change of the basal firing rate of amBNST neurons was observed before and after the HFS$_{vSUB/CA1}$ (2.74 ± 1.03 Hz versus 1.91 ± 0.74 Hz; $P = 0.401$). We next delivered HFS$_{vSUB/CA1}$ in the presence of intra-BNST infusion of the NMDA-R antagonist AP5 (100 µM). Under these conditions, HFS$_{vSUB/CA1}$ failed to elicit LTD$_{vSUB/CA1}$ (41.79 ± 13.16% of baseline, $n = 10$, $P < 0.001$; $F_{(1,15)} = 33.89$, $P < 0.0001$ for the effect of AP5 treatment × effect of HFS$_{vSUB/CA1}$ protocol interaction; $F_{(1,15)} = 28.60$, $P < 0.0001$ for the effect of the AP5 treatment; Fig. 3f). Under the same conditions, HFS$_{vSUB/CA1}$ still induced LTD$_{ILCx}$ (2.76 ± 2.76% of baseline, $n = 5$, $P < 0.001$; $F_{(1,15)} = 9.68$, $P < 0.01$ for the effect of AP5 treatment × effect of HFS$_{vSUB/CA1}$ protocol interaction; $F_{(1,15)} = 97.77$, $P < 0.0001$ for the effect of HFS$_{vSUB/CA1}$ protocol; $F_{(1,13)} = 6.90$, $P < 0.05$ for the effect of AP5 treatment, Fig. 3g). In addition, HFS protocol applied in the ILCx (HFS$_{ILCx}$) was ineffective to trigger plasticity in the ILCx nor in the vSUB/CA1 (Supplementary Fig. 3).

**In vivo LTP in the amBNST triggers anxiolysis.** Finally, we conducted behavioral experiments to test our hypothesis that vSUB/CA1-driven NMDA-receptor-dependent LTP in the amBNST triggers anxiolysis (timeline Fig. 4a), as both regions are implicated in anxiety-related behaviors. To test whether HFS$_{vSUB/CA1}$ promotes anxiolytic effect in basal but also in anxiogenic situation, we used two complementary anxiety-tests based on the innate aversion of rodents to brightly illuminated areas, with the light–dark test (Fig. 4b–f) or to open spaces with the elevated plus maze (EPM; Fig. 4e,g,h). In the light–dark test, rats exposed to HFS$_{vSUB/CA1}$ spent more time in the light compartment in basal situation compared with SHAM group (percentage of time in light: HFS$_{vSUB/CA1}$ group, 36.8 ± 3.8%; SHAM group, 27.5 ± 2.7%; $P = 0.05$, Fig. 4c). Intra-BNST AP5 infusion prevented the anxiolytic-like effect induced by HFS$_{vSUB/CA1}$; AP5 injection into the BNST decreased the % of time spent in light only in the HFS$_{vSUB/CA1}$ group ($P < 0.05$ for the effect of AP5 injection, Fig. 4c,d). Neither novelty-induced locomotor activity (Supplementary Fig. 4a) nor circadian rhythms of general activity (Supplementary Fig. 4b) were altered after HFS$_{vSUB/CA1}$ or after manipulating the activity on the NMDA receptors in the BNST, thereby reinforcing the specific role of homeostatic plasticity on behavioral anxiety. There was no significant difference in the number of transitions between groups (light–dark test: SHAM/aCSF: 15.20 ± 0.60 transitions, SHAM/AP5: 15.60 ± 1.50 transitions, respectively).
HFS VSUB/CA1/αCSF: 17.20 ± 2.20 transitions, HFS VSUB/CA1/AP5: 16.30 ± 1.50 transitions; P > 0.05) (Supplementary Fig. 4c). In the light–dark test or in the EPM, the light serves as an anxiogenic stimulus. To create an anxiogenic situation, the lighting was increased from 560 Lux to 1,230 Lux, and rats were restrained for 5 min before the light–dark test and the lighting was set-up at 260 Lux in open arms for the EPM (Fig. 4e)28. In the light–dark test, as expected, when rats were submitted to anxiogenic situation, they spent less time in the light compared with the SHAM group in basal situation (SHAM group in basal situation, 27.5 ± 2.7%; SHAM group in anxiogenic situation, 10.16 ± 3.1%, P < 0.005, Fig 4e,c). In addition, when rats are exposed to anxiogenic situation in the light–dark test or EPM (Fig. 4e–h), HFS VSUB/CA1 has still an anxiolytic effect (light–dark test: SHAM group in anxiogenic situation, 10.16 ± 3.1%; HFS VSUB/CA1 group in anxiogenic situation, 19.76 ± 4.2%; P < 0.05, Fig. 4e; EPM: percentage of time in the open arms, SHAM group: 14.95 ± 2.51%; HFS VSUB/CA1 group: 23.38 ± 3.65%; P < 0.05, Fig. 4h). No differences were observed between groups for the number of transitions in the light–dark test or EPM (light–dark test: SHAM group: 9.90 ± 0.89 transitions; HFS VSUB/CA1 group: 11.29 ± 1.60 transitions; P > 0.05, Supplementary Fig. 4d; EPM: SHAM group: 16.14 ± 1.47 transitions; HFS VSUB/CA1 group: 19.29 ± 1.96 transitions; P > 0.05, Supplementary Fig. 4e). Taken together, these data indicate that (1) HFS VSUB/CA1 decreases the steady-state anxiety level through a NMDA-receptor-dependent mechanism in the BNST (2) acute HFS VSUB/CA1 diminishes the anxiety induced by an anxiogenic situation.

Discussion
Using tract-tracing, trans-synaptic tracing from single-electroporated neurons and in vivo recordings, we report that the majority of neurons of the anteromedial BNST integrates information from the vSUB/CA1 and the ILCx at the single-cell level. Considering the differences in the straight-line distances between the ILCx-BNST (4 mm) and vSUB/CA1-BNST (6 mm), the 3 ms difference in the latency to the onset of the stimulation response between the two inputs is probably not supported by a difference in conduction velocity, but by the length of the axonal projections. We next found that HFS VSUB/CA1 triggers in vivo an evoked spike potentiation (LTP VSUB/CA1), which requires the activation of NMDA-Rs in the BNST. This HFS protocol was efficient to trigger plasticity in the amBNST when applied in the vSUB/CA1, but not in the ILCx (Supplementary Fig. 3). This is probably due to the fact that vSUB/CA1 is one of the few major output structures of the hippocampal formation and transmits learning and memory-related signals in a high-frequency bursting mode (Supplementary Fig. 2) repeated at a low frequency (0.5–2 Hz)29. A pioneering study in the hippocampus demonstrated that changes in synaptic strengths affect network activity and shape neuronal integration in an input-specific manner30. Here, we report that in response to an excessive activity of the vSUB/CA1-amBNST inputs (LTP VSUB/CA1), amBNST neurons at the single-cell level down-regulate the efficacy of their ILCx-amBNST inputs (LTD ILCx) and maintain their basal activity stable. This interaction of homosynaptic plasticity (LTP VSUB/CA1) with heterosynaptic plasticity (LTD ILCx) occurring at the single-cell level at two amBNST excitatory synapses could be considered as homeostatic plasticity15. In fact, these neuromodulatory changes correspond to a form of homeostasis necessary to maintain

Figure 3. In vivo input-specific opposite plasticity in the amBNST.
(a) Experimental protocol. (b) Cartography of recording sites in the BNST. Neurons which have been tested for the ILCx are represented in green, neurons tested for the vSUB are in magenta and those tested for both inputs are in black. (c,d) Kinetic (c) and quantification (d) of the mean percentage change (± s.e.m.) in vSUB/CA1 (magenta) and ILCx (green) evoked spike probability, normalized to the baseline, after HFS VSUB/CA1. Rmag, excitatory response magnitude. (e) Typical PSTHs (+ rasters) illustrating responses of a same single-BNST neuron before (left) and after (right) HFS VSUB/CA1 on the vSUB/CA1-amBNST pathway (top) and on the ILCx-amBNST pathway (bottom). Stimulus: 10 (gray lines). Bin width: 1 ms. Traces in insets. (f,g) Quantification of the mean percentage change (± s.e.m.) in the vSUB/CA1 spike probability (f) or in ILCx spike probability (g) in control condition or after AP5 infusion in the amBNST.
stability in the amBNST, in response to the strengthening of the vSUB/CA1-amBNST synapses (LTPvSUB/CA1) associated with a negative feedback at the ILCx-amBNST synapses (LTDILCx). Regardless of the cellular explanation, the functional effect of LTDvSUB/CA1 is complementary to that of LTPvSUB/CA1, that is it optimizes the signal-to-noise ratio by reinforcing the functional weight of the recently potentiated synapses. Interestingly, in presence of AP5 in the amBNST, the direction of plasticity at the vSUB/CA1-amBNST inputs elicited by HFSvSUB/CA1 was switch from an LTDvSUB/CA1 to an LTDvSUB/CA1 (Fig. 3f). One possibility is that AP5, locally infused in the amBNST, only partially blocks the NMDA receptors, and this partial blockade reverses the direction of plasticity elicited HFSvSUB/CA1 (refs 31,32). Another unexpected result was that the intra-amBNST blockade of NMDA-Rs potentiates LTDILCx elicited by HFSvSUB/CA1 (Fig. 3g). One possibility is that HFSvSUB/CA1 triggers concomitant activation of NMDA and metabotropic glutamate receptors in amBNST neurons, leading to a more profound LTD in the presence of AP5 (ref. 33). Further experiments are necessary to determine the molecular mechanisms by which in the absence of NMDA-Rs stimulation, HFSvSUB/CA1 triggers LTDvSUB/CA1 and potentiates LTDILCx. Finally, we can not exclude that HFSvSUB/CA1 also triggers plasticity in the mPFC23 or the basolateral amygdala34, but we provide behavioral evidence that vSUB/CA1-driven NMDA-R-dependent LTP in the amBNST triggers anxiolytic-like effects. This is in line with pioneer studies showing that changing the activity in the amBNST has a direct impact on the perception of aversive contextual stimuli35 or production of stress hormones3. In fact, here we have demonstrated, using two different anxiety assays that HFSvSUB/CA1 induced an anxiolytic effect in basal situation but also in anxiogenic situation (Fig. 4). Together, these data support the conclusion that the amBNST plays a crucial role in integrating and sending information related to anxiety9,36. Previous studies have shown that anxiety is controlled by multiple circuits in the brain, many of which share robust and reciprocal connections with the BNST4.37. These circuits include projections from the basolateral nucleus of the amygdala (BLA) to the ventral hippocampus38, from BLA to the central nucleus of the amygdala (CeA)39, from the ventral hippocampus to the medial prefrontal cortex (mPFC)40, from mPFC to BLA41 and from BLA to BNST4. Our anatomical and functional characterization of the vSUB/CA1-amBNST projection on a circuit and synaptic level further the understanding of the role played by amBNST in the modulation of anxiety3,37. In conclusion, we show that in response to HFSvSUB/CA1, homeostasis in amBNST neurons is guaranteed at the single-cell level by an NMDA-R-dependent up-scaling of the vSUB/CA1-amBNST synapses associated with an NMDA-R independent down-regulation of the efficacy of its ILCx-amBNST inputs (LTDILCx Supplementary Fig. 5). Together these findings elucidate the molecular targets that contribute to changes in synaptic functions in the amBNST, and highlight important future directions where manipulation of inputs to the amBNST using opto- or chemogenetic tools may be critical for changing network output, physiological manifestations of anxiety and anxiety-associated disorders42.

Methods

Animals. Male Sprague Dawley rats (275–300 g; 10 weeks old; Elevage Janvier, France) were used. Rats were housed three or four per cage under controlled conditions (22–23°C; 40% relative humidity, 12 h light/dark illumination cycle; lights on from 07:00 hours to 19:00 hours), were acclimatized to laboratory conditions 1 week before the experiment, with food and water ad libitum. All procedures were conducted in accordance with the European directive 2010-63-EU and with approval from Bordeaux University Animal Care and Use Committee (N° 50120205-A).

Surgery. In vivo electrophysiology. Stereotaxic surgeries for electrophysiology, tract-tracing and for light–dark test experiments were performed under 1.0–1.2% isoflurane (in 50% air/50% O2; 11 min−1 anesthesia). Stimulation electrodes, recording pipettes or injection pipettes were, respectively, inserted into the ILCx (+ 3.0 mm/bregma, 0.5 mm/midline, 4.5 mm/brain surface), the vSUB/CA1 (– 6.0 mm/bregma, 5.1 mm/midline, 7.1 mm/brain surface), the amBNST (0.0 mm/bregma, 1.3 mm/midline, 6–7.5 mm/brain surface).
Electrical stimulation of the ILCx and vSUB/CA1. Bipolar electrical stimulation of the vSUB/CA1 and ILCx was conducted with a concentric electrode (Phynex, France) and a stimulus isolator (500 µs duration, 0.2–2 mA; Digitimer, UK). Baseline was recorded for 10 min (2 × 100 pulses; 0.5 Hz). To avoid LTD, high-frequency stimulation protocol was performed in the vSUB/CA1 (HFSvSUB/CA1) at the same intensity used for baseline (0.2–1 mA). To avoid the confounding effect of epilepsy driven behavioral changes occurring in wake, freely moving animals were delivered in anesthetized animals (HFSvILCx consisted in 50 trains (500 pulses at 400 Hz, 250 µs duration pulse) presented as bursts of five trains. The frequency of the five trains was 1 Hz. Each burst of five trains was presented five times at 1 min interval (Supplementary Fig. 2).

ambNST recordings. A glass micropipette (tip diameter, 1–2 µm; 10–15 MΩ) filled with 2% pontamine sky blue solution in 0.5 M sodium acetate was lowered into the ambNST. The extracellular potential was recorded with an Axoclamp-2B amplifier and filter (300 Hz/0.5 Hz). Spikes were collected online (CED 1401, SPIKE 2; Cambridge Electronic Design; UK). During electrical stimulation of the ILCx or vSUB/CA1, cumulative peristimulus time histograms (PSTH, 5 ms bin width) of ambNST activity were generated for each neuron recorded.

Pharmacological treatment. For local delivery of 100 µM APS, double barrel pipettes were used33. For behaviour, a mixture of 180 nl of APS (100 µM) and 0.2% Fluorogold (to mark the injection site) was injected bilaterally in the BNST.

Histology. At the end of each recording experiment, the recording pipette placement was marked with an iontophoretic deposit of pontamine sky blue dye (−20 µA, 30 min). To mark electrical stimulation sites, +50 µA was passed through the stimulation electrode for 90 s. After, brains were removed and snap-frozen in a solution of isopentane stored at −80°C.

Plasmid solution. A plasmid encoding for the rabies glycoprotein, the avian virus receptor (VT) and tdTomato (tdTomato) was used in this study. For recording followed by electroporation experiments, the electrode was filled with the plasmid (pAAV-EF1a-G-TVA-tdTomato, 17.5 ng·µl−1) diluted in standard intracellular solution.

In vivo single-cell electroporation. Single-cell electroporation was performed as described previously33. After recording ambNST neurons responding to both ILCx and vSUB/CA1 stimulations, they were electroporated with a solution containing a plasmid DNA (pAAV-EF1a-G-TVA-tdTomato). We applied −10 V square-pulses delivered at 50 Hz for 1 s. Only one cell per rat was electroporated. After 2 days, an EnVα pseudotyped G-deleted rabies virus (EnvA-SAD®G-GFP) was injected into the ambNST. After 5 days, the electroporation protocol, rats were killed for immunohistological experiments.

Tract-tracing method. Tracer injections were performed as described previously33 with the following modifications. For retrograde tracing, 30 nl of 0.5% CTb (Sigma Aldrich, France) infused were used by the amBNST. For recording followed by electroporation experiments, the electrode was filled with the plasmid (pAAV-EF1a-G-TVA-tdTomato, 17.5 ng·µl−1) diluted in standard intracellular solution.

Data analysis. For in vivo electrophysiological experiments, cumulative PSTHs of ambNST activity were generated during stimulation of ILCx or vSUB/CA1. Excitatory magnitudes (Rexc values) were normalized for different levels of baseline impulse activity. Baseline activity was calculated on each PSTH, during the 500 ms preceding the stimulation. For each PSTH, Rexc values for excitation were calculated according to: excitation Rexc=−(number of spikes in excitatory epoch−mean number of spikes per baseline bin × number of bins in excitatory epoch). The cortical or hippocampal excitation strength onto ambNST neurons was determined as the amount of current needed to obtain a 50% spike probability for ILCx-evoked responses or vSUB/CA1-evoked responses (Rexc ranging from 30 to 60 pA). Results are expressed as mean ± s.e.m. Statistical analysis was performed using Student’s t-tests or Mann–Whitney when necessary. For multiple comparisons, values were subjected to a two-way Anova followed if significant by Bonferroni post hoc tests or to Kruskal–Wallis Anova for the behavioral part.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

C.G., S.C. and F.G. designed the experiments. L.M., C.X. and A.L. performed the in vivo single-cell electroporation experiments. K.Y. and B.R. made the plasmid used for electroporation. C.G., J.M., G.R.F., S.C., L.M. and D.G. collected and analyzed the data. C.G., M.D., S.C., L.M., A.L., G.R.F. and F.G. wrote the manuscript. All authors discussed the results and commented the manuscript.

Additional information

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