Subregional activity in the dentate gyrus is amplified during elevated cognitive demands

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Abstract:

Neural activity in the dentate gyrus (DG) is required for the detection and discrimination of novelty, context and patterns, amongst other cognitive processes. Prior work has demonstrated that there are differences in the activation of granule neurons in the supra and infrapyramidal blades of the DG during a range of hippocampal dependent tasks. Here we used an automated touch screen pattern separation task combined to temporally controlled tagging of active neurons to determine how performance in a cognitively demanding task affected patterns of neural activity in the DG. We found an increase in the blade-biased activity of suprapyramidal mature granule cells (mGCs) during the performance of a high cognitive demand segment of the task, with a further characteristic distribution of active neurons along the apex to blade, and hilar to molecular layer axes. Chemogenetic inhibition of adult-born granule cells (abDGCs) beyond a critical window of their maturation significantly impaired performance of mice when cognitive demand was high, but not when it was low. abDGC inhibition also elevated the total activity of mGCs and disturbed the patterned distribution of active mGCs even in mice that eventually succeeded in the task. Conversely chemogenetic inhibition of mGCs reduced success in the high cognitive demand portion of this task and decreased the global number of active GCs without affecting the patterned distribution of active cells. These findings demonstrate how a high cognitive demand pattern separation task preferentially activates mGCs in subregions of the DG and are consistent with an inhibitory role for abDGCs on the dentate circuit which in part governs the spatially organized patterns of activity of mGCs.

Introduction:

The dentate gyrus (DG) is the input structure to the hippocampus and plays important roles in episodic memory [1], spatial coding [2], novelty detection [3] as well as in separating patterns [4]. In addition to the local circuit composed of granule cells and interneurons, the neurogenic niche in the subgranular zone (SGZ) of the DG gives rise to the generation of adult-born granule cells (abDGCs) [5-8], which has been of significant interest for its potential to reorganize hippocampal microcircuits and contribute to memory flexibility [9].

While multiple functions have been ascribed to the DG, the longest standing proposal stems from early computational studies that inferred a role in pattern separation based on the anatomical connectivity of the DG [10-12]. The DG is the gateway to the hippocampus receiving direct excitatory input from the entorhinal cortex (EC). The large number of granule neurons in the DG receiving input from a relatively smaller population of EC has been proposed to allow

expansion recoding which, along with sparse firing of granule cells, allows the separation of inputs at the level of neural activity [13].

Prior work has demonstrated that mature granule cells (mGCs) localized in the two blades of the dentate gyrus termed suprapyramidal (SB) and infrapyramidal (IB) when viewed in transverse cross section [14], have an asymmetric biased activation [15-18]. More mGCs in the SB are engaged during hippocampal dependent tasks than those in the IB [15,17,18]. The divergence in mGC activity across the blades of the DG is paralleled in the asymmetry of neurogenesis in the DG with elevated numbers of adultborn dentate granule cells (abDGCs) observed in the neurogenic niche of the SB [19-22]. Despite many years of study, dissociation of the precise involvement of abDGCs versus mGCs in DG function is still not fully clear and there is no circuit-based explanation of why there is an apparent bias in mGC activity and adult neurogenesis in the DG. In this study, using TRAP2 mice [23] for temporally defined labeling of active neurons, we found that both mGCs and abDGCs were engaged during an automated spatial discrimination task. When the cognitive demand of the task was elevated by reducing the degree of spatial separation, there was an increase of mGC activity and a more prominent and amplified spatial bias with active neurons distributed preferentially to the SB and relatively reduced in the IB. Chemogenetic inhibition of abDGCs during the task decreased performance in the task. Importantly, the overall number of active mGCs was increased and the blade-biased distribution was significantly reduced suggesting that the task specific mGC neural representation are disrupted by inhibiting activity of abDGCs. Together, these findings reinforce the significance of neural activity in the DG in spatial pattern separation and highlight the circuit contributions of both abDGCs and mGCs to behaviors requiring a high cognitive demand.

Results:

Blade-biased activity of GCs during a high cognitive demand pattern separation task

Prior work has demonstrated that expression of immediate early genes in the DG including Arc [15], c-Fos [18] and Zif268 [24] correlate with behavioral state and are active in both mature and young abDGCs. Genetic strategies using TRAP1 mice [25] and TetTag mice [26,27] have also demonstrated that active GC populations can be labelled with these strategies. To determine whether behaviorally induced labeling of both mGCs and abDGCs is observed in TRAP2 mice (Fos$^{2a\text{-}iCreERT2}$;Ai9) we combined birth-dating of abDGCs with a single exposure to voluntary wheel running which is known to activate DG neurons [25] (Figure S1A). Mice given access to running wheels for 4 hours had elevated numbers of TRAP+ neurons throughout the DG, with both mature and immature neurons labelled (Figure S1B-F). TRAP+ labeling of abDGCs was observed in birth-dated neurons 14 days post injection (dpi) from BrdU (Figure S1E & F). No

double labelled TRAP+ BrdU+ neurons were observed in younger neurons prior to this timepoint (Figure S1F). Importantly, an analysis of the blade specific localization of TRAP+ neurons demonstrated that in home cage controls there was a small bias in the distribution of active neurons to the SB of the DG and this distribution of labelled GCs was not different in mice exposed to a single episode of running (Figure S1D).

In order to determine the activation patterns of GCs when mice successfully performed a high cognitive demand spatial discrimination pattern separation task, TRAP2 mice were trained in a modified touch screen task that tests the ability to separate a unique pattern of illuminated squares posing a relatively low or high cognitive demand (Figure 1A and Figure S2A & B). The trial unique nonmatching-to-location (TUNL) task has been demonstrated to require the hippocampus, particularly when the cognitive demand is high when the separation of the sample to test squares is reduced, or the delay between sample and choice is increased [28,29]. Mice were injected with BrdU to birthdate neurons at day 0 (D0). At D7 mice were water restricted before EdU injections and shaping at D14 to habituate them to the operant task and learn to touch the screen for a saccharine water reward. Mice were then trained until they reached criterion (70% correct during one of two daily sessions on 2 consecutive days) in the TUNL task with a 1s delay between the sample and choice and a large (L) separation between sample panel and the test panel (Figure 1A and Figure S2A). Mice reached criterion on average at 10.7 ± 0.8 days in the large separation sessions (Figure 1B & D left panel). After reaching criterion the mice were tested with alternating trials of large and small (LS) separation between the sample and test panels. In this case the mice were always above criterion for the L trials but took another 9.3 ± 1.0 days to reach criterion on the S trials (Figure 1C & D middle and right panel).

Different cohorts of mice were analyzed at each stage to determine the extent of TRAPed cells in the DG along with labelling for thymidine analogs which birth-dated the abDGCs at approximately 3 weeks (25 ± 1.4 days) for EdU and 5 weeks (39 ± 1.2 days) for BrdU (Figure 1E). We found a strong relationship between the stage of training in the task and the degree of activation of the mGCs with more TRAP+ neurons in mice after the completion of the L separation than during shaping, and the greatest density of TRAP+ neurons in mice after reaching criterion in the LS separation (Shape – LS, $p=0.007$)(Figure 1F). Therefore, mice that engaged in the task with a high cognitive demand demonstrated the greatest density of TRAP+ mGCs.

In the two cohorts of abDGCs that were birth-dated by BrdU and EdU we observed TRAP+ cells with only a low abundance. Approximately 1% of 25- to 39-day-old abDGCs were active (TRAP+) during the pattern separation task (LS) (Figure 1G), regardless of whether the mice reached the criterion (C) or not (NC)(Figure S2F). In contrast, in mice that only performed the low cognitive demand task (L configuration only) we observed almost no TRAP+ labelled birth-dated cells (Figure 1G). In a final group of mice, we TRAPed neurons in mice on the final day of LS trials. Mice were then reintroduced to the task (LS) 3 days later for two sessions on a single day before being perfused for immunohistochemical analysis of endogenous c-Fos expression (90 min after behavior, Figure 1A). Performance of mice in the task was diminished compared to the last day of prior training in both the L and S trials (Figure 1D, middle panel). Assessment of re-activation of mGCs (i.e., TRAP+ cells expressing endogenous c-Fos after the last session) was measured in these mice, with a larger overlap in TRAP+/c-Fos+ cells for mice who performed the LS trials than in mice that only performed L separation trials ($p = 0.05$) (Figure 1H). Collectively, our results demonstrate that performing a high cognitive demand task of visual pattern separation increases the activation of DG granule cells including both mGCs and abDGCs.

To determine whether performance of the high cognitive demand pattern separation task altered the spatial profile of active mGCs we conducted an analysis of the spatial distribution of TRAP+ mGCs in the DG (Figure 2). After performing the high cognitive demand LS task there was a marked spatial bias of TRAP+ cells in mice with active neurons distributed preferentially to the SB than the IB in the DG ($p= 0.0282$) (Figure 2A). A further analysis of active neurons in the DG (TRAP+) to determine the location in relation to the hilus and dentate apex demonstrated that active cells were more prominent in the outer radial areas of the granule cell layer (GCL) a region that has an enrichment in spatially biased semilunar granule cells [17,30], and closer to the DG apex in mice after the more demanding LS task (Figure 2B & C). These data demonstrate that there is biased activity of suprapyramidal granule neurons during the performance of the high cognitive demand segment of the TUNL task, and that there is a pronounced difference in the distribution of the active neurons along the apex to distal blade and the hilar to outer GCL axes (Figure 2D & E).

Activation of GCs during remote recall in the high cognitive demand task

We next investigated whether a similar spatially biased activity of GCs was observed in a remote recall (R) test three weeks after reaching the criterion during the initial training (I), and whether there was overlap in the population of active DG neurons (Figure 3A). In the remote

session, mice had a high success rate performing close to criterion in the L trials on the first day of retesting suggesting that 3 weeks after initial learning they retained the ability in task (Figure 3B). However, when confronted with the more demanding trials with inclusion of the small separation configuration, mice required additional days to achieve the criterion level (Figure 3C), although most mice required less time to reach criterion in LS training during the remote trials than on the initial testing (Figure 3D). We again observed that the density of TRAP+ cells was increased in mice that underwent LS compared to L only trials during the initial training (not shown) and importantly the distribution of active neurons during both the initial training (TRAP+) and remote training (c-Fos+) were biased to the SB after LS training (Figure 3F & G).

An analysis of birth-dated active neurons labelled during the initial training (TRAP+) or during the remote trials (c-Fos+) demonstrated a small number of BrdU (~4 weeks) neurons were labelled during the initial training (Figure 3H) whereas in the remote training we observed both BrdU labelled (7.7 weeks) and EdU labelled (5.7 weeks) abDGCs that were c-Fos+ only in mice after LS training (Figure 3I). No birth-dated abDGCs were found that were double labelled TRAP+ and c- Fos+, however we observed dual labelled mGCs with more TRAP+/c-Fos+ labeled neurons in mice that had performed LS training than those that only performed L training ($p=0.0433$) (Figure 3J).

Taken together, these results demonstrate that during initial training and remote training in the pattern separation task, spatially biased activity of neurons is observed in the SB but there is little overlap in activity of neurons in the DG of mice during these epochs of training.

DREADD inhibition of abDGCs disrupts blade-biased activity of mGCs and performance of mice in a high cognitive demand task

abDGCs are known to affect the excitability of mGCs [31,32] and there is a demonstrated blade bias in neurogenesis in the DG. However, it remains unknown whether abDGCs affect the blade-biased activity patterns of mGCs that are evident during a pattern separation task. To acutely modulate the activity of abDGCs during task performance, we created mice in which the inhibitory DREADD (designer receptor exclusively activated by designer drugs) could be conditionally expressed in abDGCs by crossing R26-LSL-Gi-DREADD mice (hM4Di) [33] to Ascl1^{CreERT2} mice. This selectively expresses the hM4Di inhibitory DREADD in a birth-dated cohort of abDGCs by the delivery of TAM (tamoxifen, see Methods) in the diet of mice for 7 weeks allowing the inhibition of their activity with a selective ligand deschloroclozapine (DCZ)(50 $\mu g.kg^{-1}$)[34] during the LS sessions of the task (Figure 4A & B) [35,36]. Inhibition of abDGCs (≤ 7 weeks old) using the DREADD ligand DCZ increased the time animals took to

reach criterion compared control groups of animals that received DMSO or were Cre negative (Cre-) ($p = 0.01$ and $p = 0.05$) (Figure 4C & D). This impairment was specific to the cognitively more demanding LS task, as inhibiting ≤ 7-week-old abDGCs did not affect the performance of mice once they had learned the L configuration (not shown). Importantly control groups that had also received TAM in their diet showed no effect in the pattern separation task or in the biased blade activity of mGCs (Figure 4C-J). Taken together this experiment suggests that abDGCs in this cohort (≤ 7 weeks) are beneficial to performance in a pattern separation task.

Prior work has demonstrated that abDGC loss causes an increase in activity of mGCs [31,37] which is likely through a loss of connections to hilar interneurons which provide feedback inhibition to the DG. Consistent with this, analysis of c-Fos in the DG demonstrated increased labelling across the DG when abDGCs were inhibited with DCZ activation of hM4Di compared to control groups ($p=0.0271$) (Figure 4E & F). Conversely, c-Fos+ labeling in the downstream CA3 region of the hippocampus demonstrated no significant difference in density between the control groups and abDGC inhibition (Figure 4G). Interestingly, we found that the biased distribution of c-Fos+ neurons in the two control groups showed the same profile as we had observed before in the IB and SB after mice perform the high cognitive demand task (Figure 4H). However, in the cohort of mice in which abDGCs were inhibited, the prominent biased distribution pattern of activity was not observed ($p = 0.01$) (Figure 4H). Furthermore, the distribution of c-Fos+ cells within the blades showed that when abDGCs were inhibited during LS training there was a marked prominence of labelled neurons located near the hilus in both the IB and SB in the region closest to the subgranular zone (SGZ), the neurogenic niche of the DG where abDGCs are produced, raising the possibility that these neurons are under greater feedforward inhibitory control via abDGCs (Figure 4I). In summary, DREADD inhibition of ≤ 7-week-old abDGCs during the LS high cognitive demand task resulted in a reduction in both in biased SB-IB spatial activity pattern as well as the activity labeling of neurons along both axes of the transverse DG (Figure 4J), and this altered activity correlated with a reduction in performance of animals which required increased days to complete the high cognitive demand task.

In the previous experiments we provided TAM to mice in their diet to cause expression of hM4Di in neurons that range from 0 days to 7 weeks. This covers abDGCs that are within their critical period when they demonstrate strong plasticity and excitability and are well connected to the hippocampal network [38,39]. To determine if DREADD inhibition of a population of younger neurons had any effect on the pattern separation task we supplied TAM in the diet for 4 weeks

to express hM4Di in abDGCs ≤ 4 weeks (Figure 5A). In the LS high cognitive demand task, there was no difference in the time to criterion of animals that had received DCZ administration for hM4Di inhibition of abDGCs compared to the control groups (Figure 5B). Similarly, analysis of c-Fos+ density in the DG found no difference in overall density (Figure 5C) and the blade distribution of active mGCs after hM4Di (Figure 5D) or in the hilar to molecular layer axes distribution (Figure 5 E).

Taken together these results demonstrate that ≤ 7-week-old cohort of abDGCs that include neurons in their critical period of development influence the excitability and spatially biased pattern of activity of mGCs in the DG and also affect the performance of mice in a high cognitive demand pattern separation task. In contrast young neurons at ≤ 4 weeks do not influence either the performance of mice in the task or the spatial distribution of activity in the DG.

Both the increase of mGC activity and the blade-biased distribution are necessary to perform in high cognitive demand task.

Our results have confirmed that the activity of mGCs, which can be regulated by abDGCs, is required for the efficient performance in a high cognitive demand pattern separation task. mGCs are under strong inhibitory control and are sparsely active in vivo [40,41]. To assess how artificially affecting their activity with an inhibitory DREADD we crossed Dock10-Cre mice [42] with R26-LSL-Gi-DREADD mice to express the hM4Di in mGCs and assessed their performance in the high cognitive demand task (Figure 6A). As we had predicted, DREADD inhibition of mGCs caused a majority of mice to not succeed in reaching criterion (62.5%) within the 14 day LS cutoff we imposed compared to mice in the control group (34.8%)(p=0.0452)(Figure 6B & C). Performance in the interleaved low demand L trials was not degraded by mGC DREADD inhibition (not shown). There was a clear reduction in total c-Fos+ labeling in the DG (p = 0.04) and CA3 (p = 0.006) of mice that did not achieve criterion after 14 days (Figure 6D & E). Surprisingly despite mice not reaching criterion in the LS task and reduced overall activity there was a biased distribution of active c-Fos+ labeled neurons to the SB which was no different to control mice that reached criterion suggesting that both the overall excitability and biased distribution of mGC activity are essential for a high cognitive demand pattern separation task (Figure 6F-H).

Discussion:

In this study we used activity labelling techniques combined with a complex, automated visual spatial pattern separation task to capture patterns of activity of GCs in the DG. The DG is a critical part of the hippocampal formation that receives extrinsic input from cortex and other

structures but is also highly regulated by local connections with hilar and CA3 neurons[13]. While many roles have been ascribed to the DG, a prominent theory has been that its activity is vital to pattern separation [43]. We found that activity labeling of neurons demonstrated unique spatial distributions of active GCs which were amplified when the separation task was made more stringent for the mice. In particular we observed a biased distribution of active neurons in the SB which was further elevated after mice successfully completed the LS task. There have been prior descriptions of a blade biased distribution of active neurons when mice engage in hippocampal dependent behaviors [15-18] however it is still not known how these spatially defined activity patterns are modulated or established. We found that not only is there a bias towards more activity in SB and less in the IB during a more demanding task, there is also an increase in density outer radial areas of the granule cell layer (GCL) where semilunar granule cells are enriched [17,30], and in regions closer to the dentate apex. These biased spatial distributions of active neurons were not observed when mice were assessed after running, a behavior that increases activity of mGCs significantly but does not enhance their blade-biased distribution.

abDGCs in dentate function

The dentate gyrus also, uniquely for a temporal lobe structure, has a neurogenic niche where new neurons are born and integrate into the existing network in the dentate. Post mitotic abDGCs develop over the course of several weeks [44], reaching maturity by around 8 weeks post mitosis where their functional properties and connectivity resemble those of other mGCs [45,46]. Prior to this, during a critical period of their development when neurons are between 4-8 weeks post mitosis, it has been proposed that the cellular properties and connectivity in the microcircuit imbue unique roles for abDGCs, functionally distinguishing them from mGCs [47], including involvement in behaviors that require discrimination of similar contexts [48,49]. Notably, it has been proposed that abDGCs affect the local circuit by contributing to the sparsity of mGC activity through feedback inhibition [47,50,51] or through direct inhibition [52]. Transformations of cortical inputs to sparse representations in the DG are a prerequisite for the distributed code required to separate similar input patterns. Behavioral experiments support the involvement of the DG in pattern separation [53]. Manipulations that ablate abDGCs cause impairments in spatial discrimination when the task is made more challenging with less separation between the locations [54]. Conversely genetic or environmental manipulations aimed at increasing neurogenesis enhance the ability of animals in spatial discrimination tasks [48,55]. In our experiments we detected limited numbers of labeled abDGCs during performance of a task as might be expected given that the population of these neurons is much lower that mGCs. However, we did observe that older neurons that were ~7 weeks post mitosis were more

prominently labeled compared to ~4 week postmitotic neurons as might also be expected given these are more connected to the local network. Importantly these neurons had an outsized influence on the DG. We used an acute and reversible method to chemogenetically inhibit different cohorts of abDGCs just during task performance [36], and similar to studies which have made chronic manipulations of neurogenesis [31] found that there was a general increase in active mGCs. More importantly acute inhibition of abDGCs during the LS task reduced performance of mice in the high cognitive demand discrimination trials without degrading their performance in already established low demand discrimination trials in the same sessions. Analysis of active labelled mGCs in mice that successfully navigated the task but with reduced performance demonstrated a correlated effect on the spatial distribution of labelled neurons in the DG. The enhanced SB to IB biased activity of mGCs was not observed. In addition, the characteristic distribution of mGCs along the hilar to molecular layer axis of the DG and from the dentate apex to the blade tips when mice performed the high cognitive demand task was also not observed when the abDGCs were chemogenetically inhibited. Again, these effects were only observed when cohorts of $\leq$ 7-week-old abDGCs were targeted with the DREADD but not when cohorts of $\leq$ 4-week-old abDGCs were inhibited. Therefore, our results reveal an important role for abDGCs in affecting dentate activity patterns and behaviors that impose a higher cognitive demand likely through this modulatory effect on the DG rather than serving as direct encoders of information [56]. This is consistent with a model where the required activity representation in the dentate for efficient pattern separation is influenced by cohorts of abDGCs as they are maturing through their critical period by both sparsening and influencing the spatial distribution of active mGCs.

Activity levels of mGCs is required for a high demand task

In our final experiment we used an acute and reversible DREADD inhibition of mGCs using a Cre driver line expressed in mGCs but not abDGCs (DCX positive neurons, data not shown). mGCs are sparsely active in vivo [57] which is a key feature of dentate circuit function. However, imposing a chemogenetic suppression of their activity during the LS task had a major effect on the performance of mice with a majority of the tested cohort not reaching criterion. Analysis of these mice demonstrated that during S trials they remained at chance whereas they continued to perform above criterion at interleaved L trials (not shown). Consistent with inhibition of mGCs activity, c-Fos+ neuron density was reduced in the DG, however surprisingly the blade biased distribution of active neurons was evident in mice that performed the task but were not successful in reaching criterion. Thus, unlike with abDGC inhibition experiments there was not a clear correlation between effects on behavior in the LS task and the spatial distribution of active

cells. This demonstrates that the effect of direct inhibition of mGCs produces a fundamentally different result on the activity of the circuit that affects the behaviors accordingly. While for abDGC DREADD inhibition slowed performance in the successful completion of the task, even in mice that reached criterion there was altered spatial distribution of active mGCs. In the case of direct inhibition of mGCs overall excitability was altered but the spatial pattern of activity was not in both mice that did or did not reach criterion (not shown). Moreover, the subset of mice that did reach criterion did so in a time indistinguishable from the control cohort (not shown). Prior studies using genetic manipulations to increase neurogenesis, have identified blade-specific changes in the number of c-Fos+ cells, with an increase in the IB following contextual discrimination [58], and a corresponding increase or decrease in the IB or SB respectively, after navigational learning [59]. These findings support the hypothesis that the SB is context-specific and involved in pattern separation, while the IB is more associated with spatial representation and memory precision, a notion further corroborated by our study[56].

Limitations of the Study

The use of activity tagging has the inherent limitation that it provides a snapshot in time of activity during a window and more nuanced effects of activity patterns during the behaviors are not captured. This is countered by the less invasive nature of these experiments than ones that require implants for in vivo recording of neuron activity. Future studies using in vivo measurements of activity will be required to support the present findings and find conclusive neural representations required for discrimination of similar patterns.

Materials and methods

Ethics statement: All animal procedures were in accordance with use protocols approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC). Male and female mice were housed 3–5 per cage on a reverse light/dark cycle of 12/12 hours within a controlled environment. They received food and water *ad libitum*. All behavioral testing was conducted during the dark period for the animal and performed blind to the genotype. Tail biopsies were used to perform genotyping through PCR of all mice used in the study. All efforts were made to reduce the number of mice used and to minimize any pain or stress.

Animals: All mouse lines were maintained on a C57Bl/6 background. Mice were propagated by breeding females heterozygous for Cre recombinase and homozygous for the Ai9 allele (Rosa-CAG-LSL-tdTomato-WPRE) or the hM4Di allele (R26-LSL-Gi-DREADD) with male mice homozygous for Ai9 or hM4Di allele only. TRAP2;Ai9 mice were generated by crossing $Fos^{2A\text{-}iCreER/+}$ (TRAP2) mice (Jax #030323)[23] to Ai9 (Jax #007909) mice to obtain the double

heterozygous mice. Ascl1-CreER^{T2} ; hM4Di mice were generated by crossing R26-LSL-Gi-DREADD mice (Jax #026219) [33] to mice containing tamoxifen-inducible Cre recombinase under the control of the *Ascl1* promoter (Jax #:012882) [60] to produce double-transgenic progeny Ascl1-CreER^{T2};R26$^{LSL-hM4Di}$. Cre-negative littermates from heterozygote breedings were used as controls. Dock10-Cre; hM4Di mice were generated by crossing Dock10-Cre [42] to the hM4Di mice to produce double-transgenic progeny Dock10-Cre;R26$^{LSL-hM4Di}$.

Birth-dating of abDGCs: 6–8-week-old mice received three intraperitoneal (i.p.) injections of 5-Bromo-2′-deoxyuridine (BrdU, Sigma Aldrich #B9285) at 100 mg/kg (i.p) at 4 h intervals on a single day [61]. 5-Ethynyl-2′-deoxyuridine (EdU) injections were administered 2 weeks after BrdU using the same injection protocol (Sigma Aldrich, #900584).

Tamoxifen administration: *IP injections:* To time the labelling of TRAP+ cell in TRAP2 mice, 4-hydroxytamoxifen (4-OHT; Sigma Aldrich, #H7904) was administered intraperitoneally. 4-OHT was dissolved at 20 mg/mL in pure ethanol and stored at –20°C for up to several weeks. Before use, 4-OHT was redissolved in corn oil (Sigma, Cat #C8267), shaken at 90°C until the ethanol had evaporated to obtain a final concentration of 10 mg/mL 4-OHT. The final 10 mg/mL 4-OHT solutions was used on the day it was prepared. All injections were delivered intraperitoneally at 50 mg/kg at the end of the training phase of the day or after 2 hours of running.
Oral administration: For conditional expression of hM4Di in Ascl1 positive cells, tamoxifen diet (TD.130858, Envigo) was given *ad libitum* to the mice for 4 or 7 weeks depending on the experimental timeline. Body weight was carefully monitored every day during the experiment. The food contained 500 mg tamoxifen/kg of the chow which provided ~80 mg tamoxifen kg^{-1} body weight per day, assuming an average body weight of 20-25 g for the mice and 3-4 g intake.

Inhibitory DREADD-hM4Di activation: Deschloroclozapine (DCZ, MedChemExpress #HY-42110) was dissolved in 2% DMSO in Saline 0.9% and administered by i.p. injections of 50 $\mu g.kg^{-1}$ [34]. Behavioral trials started 30 minutes after the i.p. administration of the drug.
Behavioral procedures: Adult (8-10 weeks-old) male and female mice were used in behavioral studies. Before the beginning of behavioral paradigms all animals were handled 2 min per day for 3 days by the experimenter.

Running wheels: To habituate the mice to the running wheel and to running, wireless running wheels (ENV-047, MedAssociates) were added to the home cage for 5 days (5 mice per cage max). Two days before BrdU injections, running wheels were removed from the cage. One week before the experiment mice were individually housed. On the day of the experiment, a running

wheel was added in each cage for a duration of 4 hours. After 2 hours, mice received a 4-OHT injection and were then returned to their respective cages. Following an additional 2 hours of running, the wheels were removed from the cages. Three days later, wheels were reintroduced to the cage and remained until mice were euthanized for assessment of TRAP+ cells, 90 minutes after the beginning of the run. The effect of exposure to the wheels was quantified by measuring the total distance run by each of the mice.

Automated touch screen operant behaviors: Testing was conducted in custom-built, touch screen-based automated operant device boxes built specifically for mice. The apparatus consisted of a rectangular modular testing chamber (w14.5cm x l21.5cm x h18.5cm). A 60mW green LED was located above a port that gave access to a retractable water bottle at the back of the chamber. At the opposite of the chamber, a flat-screen infrared touch monitor was controlled by custom scripts (Python). In front of the touch screen there was a mask with 2 horizontal lines of 5 squares. Mice performed a modified version of the “trial unique nonmatching-to-location” (TUNL) task [29]. Five days prior to testing and during the training phase, mice were placed on a water-restriction regime in which they had access to water for 10 minutes per day and were maintained at 85-90% body weight during the entire duration of the experiment. Mice were trained each day, in two separate sessions of 30 trials or a maximum of 30 minutes. Experiments started with “Shaping” in which following habituation to the operant chamber for 30 minutes, mice were trained to touch a lit white square stimulus presented randomly in one of the response windows of the touch screen in order to receive a reward (2 second water nozzle access of saccharin water 0.01%). Mice were trained in Shaping until they successfully achieved 30 rewards within 30 min during 3 successive sessions. Mice were then transitioned to training in the TUNL task with a Large-only separation (L configuration). For this task, each trial was composed of two phases. During the sample phase, one white square was illuminated in one of the 10 possible locations on the screen (Figure S2A & B). Following a nose-poke to the sample square the stimulus disappeared, and the mouse received a reward only on 33% of the trials. After a 1s delay from the sample trial, the choice trial was initiated where two stimuli were presented one in the repeated sample location (incorrect) and the other in the new location (correct) separated spatially by 4 squares in the L configuration (Figure S2A & B). A touch to the correct sample location resulted in delivery of a reward and an inter-trial interval (ITI) of 5s before the next trial was initiated. An incorrect choice resulted in a 10 second punishment (roof lights turned on) and then an ITI, followed by correction trials in experiments during the first two days of training. Correction trials followed the same procedures, except that the same sample and choice locations from the previous incorrect trial were presented again

until the correct choice was made. The correction trials were not included when calculating performance. After successfully completing 30 trials within 30 minutes at over 70 % correct for at least two out of four sessions on two consecutive days in the Large configuration, the mice were transitioned to the next phase of the TUNL task with a combined sessions with presentation of stimuli with both Large separation and Small separation between sample and choice squared (LS configuration). The training followed the same pattern as in the previous session except that there are two separate possibilities for the presentation of the choice square which was either separated by 4 squares from the sample square during L trials or the choice square was 0-1 square separated from the sample location during S trials (Figure S2B). Mice trained in the LS phase with 15 of each alternating L and S trials until they reached criterion which was set at 70 % successful S configuration trials in two out of four sessions on two days.

Immunohistochemistry: Mice were deeply anaesthetized with isoflurane and then perfused transcardiacly with PBS containing 0.02% sodium nitrite followed by 2% paraformaldehyde (PFA) in 0.1 M sodium acetate buffer (pH 6.5) for 3 min, and 2% PFA in 0.1 M sodium borate buffer (pH 8.5) for 10 min [62]. Brains were dissected and post-fixed in 2% PFA 0.1 M sodium borate buffer (pH 8.5), for 24-48 hours and stored at 4°C in PBS until sectioning. 50µm floating sections were collected on a Leica Vibratome VT1000 and stored at 4°C in PBS containing 0.02% sodium azide. For c-Fos analysis, perfusions were performed 90 minutes after the end of the behavioral training. Immunohistochemistry using standard procedures was conducted at room temperature. Free-floating sections were incubated for 30 min in a solution of 25 mM of Glycine to decrease the background signal and then rinsed two times in Tris-Buffered Saline 1X (TBS). Sections were then blocked for 1 hour in the saturation solution containing TBS-Triton (0.25% Triton X-100 (Fisher, BP151-100), 3% normal donkey serum (NDS; Jackson ImmunoResearch, 017-000-121) and 0.1% bovine serum albumin (BSA; Sigma, A9085)). The sections were then incubated overnight in the saturation solution containing the primary antibody (rabbit anti c-Fos (1/1500, Cell Signaling), sheep anti-BrdU (1/2500, NovusBio), rabbit anti RFP (1/5000, Abcam)). For c-Fos staining, no serum and BSA were used. For BrdU staining, sections were incubated 10 min in a solution of DNAse I (Sigma #D5025, 15 KU/mL dissolved in 0.05 M NaCl), $MgCl^2$ (6 mM) and $CaCl^2$ (10 mM) to cleave DNA then rinsed 2 x 5 min in TBS before the saturation part. For EdU staining, sections were incubated in solution from the kit ClickIT™ (#C10340 Thermofisher) during 30 min then rinsed 2 x 5 min TBS before the saturation part. No antibodies were necessary for the rest of the immunostaining. The next day, sections were washed 2x5 min in TBS before being incubated for 1 hour in secondary antibody solution (donkey anti-rabbit Alexa 647 or 488 (1/1000, Invitrogen), donkey anti-sheep

Alexa 488 (1/1000, Invitrogen)). Finally, sections were washed 2x5 min in TBS and cover-slipped with ProLong Diamond mounting media 275 containing DAPI (#P36962, ThermoFisher Scientific).

Imaging and neuron quantification: Confocal images were obtained with a Zeiss confocal system (LSM700) with a x20 objective with the experimenter blind to the genotype and condition following previous protocols [61]. One section in every ten serial sections were analyzed through the dorsal hippocampus (stereotaxic coordinates: -1.20 to -2.30 relative to bregma according to Paxinos' brain atlas). Two to three 10 µm z-stack acquisitions, were made to picture the whole dentate gyrus. 3D reconstructions were used to univocally verify co-localizations (i.e. activated mature neurons or immatures neurons). All acquisitions were carried out in sequential scanning mode to prevent cross-bleeding between channels. Stacks of images were then reconstituted using Fiji software, allowing the experimenter to count the number of cells. The surface area of the dorsal hippocampus, DG, SB, IB and CA3 were traced in mapping software (Neurolucida Zeiss) using a x10 objective. The volume was determined by multiplying the surface area by the distance between sections (500 µm). Densities of BrdU+, EdU+, TRAP+ and c-Fos+ cells were estimated by multiplying the total number of labelled cells by 10. Four to seven mice per group were used for these analyses.

Distribution of TRAP+ and c-Fos+ Cells in the DG: We followed methods presented in literature [63]. Using stacks of images obtained from confocal microscopy, the localization of TRAP+ and c-Fos+ cells in the DG was assessed using Fiji software. *Distance from the hilus:* The contours of each blade were manually outlined and the distance from the center of each cell body to the hilus was manually measured. Cells located in the apex region were excluded from the counting.

Distance from the apex: Excluding the apex region, the size of each suprapyramidal blade was measured as well as the distance of each cell from the apex. These distances were then expressed as percentage of total blade size.

Statistical analysis: Data are expressed as means ± S.E.M. Statistical analysis was performed using Statview and Origin programs. For behavioral experiments, one sample *t*-tests were used to compare performance against chance (50% in the TUNL experiment). For histochemical data, we used independent comparisons with Mann–Whitney U test for non-paired samples (non-parametric). To test more than two independent samples we used the Kruskal Wallis test. In case of significance, a post-hoc pairwise comparison (Dunn's test) was made to identify the group that differed. Differences were considered to be significant when $p < 0.05$.

Figure Legends:

Figure 1 : **TRAP labeling of mGCs and birth-dated abDGCs after the TUNL pattern separation task**

(A) *Top:* Schematic illustrating the pattern separation paradigm. Male (n = 9) and female (n = 7) mice (6-8-week-old) received 3 injections of BrdU at D0 and 3 injections of EdU two weeks later (D14) before starting the behavioral paradigm. Mice received one injection of 4-OHT on the day they reached criterion and were perfused 3 days later 90 minutes after one exposure to the behavioral paradigm to allow endogenous c-Fos expression. Control mice (males n = 6, females n = 4) underwent the same treatment but only participated in the Large separation task (L). Mice were water restricted during the behavioral experiment. *Bottom:* Cartoon representation of the chamber's in TUNL task with examples of Large separation (L) and Small (S) separation configurations. High cognitive demand paradigm consisted of interleaved L and S trials (LS).

(B) Number of days for each mouse to reach the 70% success criterion in the Large separation (L).

(C) Number of days for mice to reach 70% success criterion in S trials, during the LS separation task.

(D) *Left*: Average percentage of correct choices in the L separation on the first day of training, as well as during the three days prior to reaching criterion (days before criterion, DBC) and the day of reaching the criterion (crit)(one-sample *t*-test to chance at criterion Large $t(16)=12.04$, $p<0.0001$). *Middle*: Average percentage of correct choices in the L and S separation on the first day of training, during the three days before criterion (DBC), on the day of reaching criterion and 3 days later (one-sample *t*-test to chance at criterion Large $t(16)=8.38$, $p<0.0001$; Small $t(16)=7.37$, $p<0.0001$). *Right:* Cumulative probability of days to criterion of mice in S for both L and LS groups.

(E) Example section of BrdU+ labeling (green) EdU+ labeling (blue) and TRAP+ labeling (red) in TRAP2 mice. Calibration bar: 30 µm.

(F) Density of TRAP-labeled GCs in mice undergoing LS compared to control mice that were engaged in either shaping (Shap) or the Large configuration task only (L) ($H(2) = 7.42$, $p = 0.0245$; Kruskal Wallis)(With Dunn post hoc analysis: Shape comparison to L, $p = 0.112$, Shape comparison to LS, $p=0.007$, L comparison to LS $p = 0.194$).

(G) Percentage of BrdU labeled cells (39 days +/- 1.23) or Edu labelled cells (25 days +/- 1.37 days) also TRAP+ after L or LS training (BrdU+/TRAP+ L: 0.145 ± 0.145 %, 6 mice; LS BrdU+/TRAP+ 0.822 ± 0.474 %, 6 mice; ns; EdU+/TRAP+ L: 0.203 ± 0.144 %, 6 mice; LS BrdU+/TRAP+ 0.304 ± 0.155 %, 7 mice; ns; Mann–Whitney).

(H) Percentage of TRAP+ GCs expressing c-Fos+ in the L or LS task after reaching criterion (expressed as a percentage of TRAP+ cells) (L TRAP+/c-Fos+: 1.578 ± 1.119 %, 4 mice; LS TRAP+/c-Fos+: 3.926 ± 2.277 %, 6 mice; p = 0.05; Mann–Whitney).

Figure 2: **Blade-biased activity of mGCs during a high cognitive demand pattern separation task**

(A) Spatial distribution of TRAP-labeled cells in the infrapyramidal (IB) and suprapyramidal (SB) blades of the dorsal DG in TRAP2 mice performing the LS separation, compared to control mice that performed only the L configuration. TRAP+ neurons showed a preferential distribution to the SB over the IB in the DG, with a more pronounced bias in the LS group of mice (IB : L: 43.410 ± 1.51 %, 6 mice; LS : 34.01 ± 2.79 %, 8 mice; p= 0.0282; SB : L: 56.56 ± 1.51 %, 6 mice; LS : 65.99 ± 2.79 %, 8 mice; p= 0.0282; Mann–Whitney)

(B) Spatial distribution of TRAP+ cells in the hilar to outer radial GCL axes in the SB (0 to 120µm) and the IB (0 to 120µm) of the dorsal DG with 0 µm indicating location at the hilar border.

(C) Distribution of TRAP-labeled cells along the apex to blade extremity axes of the SB of the dorsal DG, with 0% indicating location at the dentate apex (L: 0-50: 51.9 ± 2.18 %, 6 mice; 50-100: 48.1 ± 2.18 %, 6 mice; ns ; LS : 0-50: 56.0 ± 2.44 %, 6 mice; 50-100: 43.8 ± 2.54 %, 6 mice; p= 0.0065; Mann–Whitney).

(D) Example section of TRAP+ cells in the dorsal DG illustrating the distinct boundaries between the two blades, the hilus, and the apex. Calibration bar: 20 µm.

(E) Cartoon illustrating the two blades of the DG: IB and SB. Dentate granule cells are depicted in grey while red circles represent TRAP+ cells in the DG. In the L group, a greater number of labelled cells are localized in the SB compared to the IB. This bias of activity is also observed in the LS group, where the bias is more pronounced, and the overall activity of the DG is increased. In the SB, the majority of the activated cells are located closer to the apex region.

Figure 3: **Activation of GCs during remote recall in the high cognitive demand task**

(A) Schematic representation of the pattern separation paradigm used in the study. Mice (male n=11, female n=8) performed two trainings sessions: initial (I) and remote (R). The second training (R) took place 3 weeks after the mice reached the S criterion in LS. Mice were perfused when they reached the criterion in S during R. A second group that performed only in the L configuration during both training sessions was included as control (males n=3, females n=3).

(B) Learning curves representing the percentage of success during the remote test (R) for the L (green) and LS (orange) groups (one-sample *t*-test to chance at criterion; LS: Large $t(14) = 11.8$, $p < 0.0001$, Small $t(14) = 10.3$, $p<0.0001$; L: Large $t(3) = 6.78$, $p = 0.0066$).

(C) Percentage of mice reaching the L and S criterion in LS group, during the remote test (R) represented as cumulative probability. During the remote test, mice reach the L criterion in just a few days but require more time to reach the S criterion.

(D) Performance of individual mice indicating a non-significant reduction in the number of days needed to reach the criterion between the initial and remote training sessions. Only 4 mice (red lines) required more days to reach the criterion during the remote test. (I: 8.067 ± 1.173 %, R: 5.93 ± 0.753, 15 mice; p= 0.204; Mann–Whitney).

(E) EdU labeled cell that was also TRAP+ labeled after the initial training but was that was not reactivated (c-Fos) during the remote test. EdU (green), TRAP (red), c-Fos (blue) in TRAP2 mice. Calibration bar, 10 µm.

(F) Distribution of TRAP-labeled cells in the IB and SB blades of the dorsal DG in TRAP2 mice. Labeling was performed in LS separation during initial training (I) and compared to control mice that performed only the L configuration. Active neurons show a preferential distribution to the SB over the IB in the DG. This bias is more prominent in the LS group of mice (training I: IB : L: 47.3 ± 3.312 %, 4 mice; LS : 33.7 ± 3.31 %, 4 mice; p = 0.021; SB : L: 52.7 ± 3.32 %, 4 mice; LS : 66.4 ± 3.31 %, 4 mice; p= 0.021; Mann–Whitney).

(G) Distribution of c-Fos labeled cells between the IB and SB blades of the dorsal DG in TRAP2 mice performing the LS separation during remote training (R), compared to control mice that performed only the L configuration. Active neurons show a preferential distribution to the SB over the IB in the DG (LS, IB : 31.4 ± 3.11 %, 4 mice; SB : 68.7 ± 3.11 %; p=0.021; Mann–Whitney). The bias of activity is similar between the two groups (R, IB : L: 41.1 ± 4.18 %, 4 mice; LS : 31.4 ± 3.101 %, 4 mice; p= 0.191; SB : L: 58.9 ± 4.18 %, 4 mice; LS : 68.7 ± 3.11 %, 4 mice; p= 0.191; Mann–Whitney).

(H) Percentage of newborn neurons (BrdU (7.7 weeks) or EdU (5.7 weeks)) activated during the initial training (I), as indicated by co-labeling of $TRAP^+$(BrdU+ c-Fos+: L: 0.242 ± 0.242 %, 6 mice, LS: 0.170 ± 0.170 %, 6 mice; ns ; Mann–Whitney).

(I) Percentage of newborn neurons (BrdU or EdU) activated during the remote test (R), as indicated by c-Fos expression (BrdU+ c-Fos+: 0.977 ± 0.426 %, 6 mice, p=0.05; EdU+ c-Fos+: 0.694± 0.317 %, 9 mice; p= 0.0785; Mann–Whitney).

(J) Percentage of mGCs activated during the (I) training ($TRAP^+$) that were reactivated during the R test (c-Fos+) (expressed as a percentage of TRAP+ cells) (TRAP+ c-Fos+: I: 0.764 ± 0.309 %, 4 mice; R: 1.68 ± 0.067 %, 4 mice; p= 0.0433; Mann–Whitney).

Figure 4: **DREADD inhibition of ≤ 7-week abDGCs during a high cognitive demand task**
(A) Schematic representation of the pattern separation paradigm. Prior to onset of the training, mice (males n = 17, females n = 16) were provided tamoxifen-containing food ad libitum in the home cage for 7 weeks to activate TAM inducible Cre recombinase (Ascl1-$CreER^{T2}$) in abDGCs. All mice received DCZ (deschloroclozapine, 50 $\mu g.kg^{-1}$) or DMSO injections 30 minutes before the beginning of LS trials.
(B) Example section from Ascl1-$CreER^{T2}$; hM4Di mice after LS training. c-Fos+ neurons are labeled in red, TAM induced neurons expressing citrine are labelled green. Calibration bars *top*: 140 µm *bottom*: 50 µm.
(C) Percentage of mice reaching the criterion in the S trials across days represented as the cumulative probability. The Cre+ DCZ treated group needed additional days to reach criterion compared to controls.
(D) Number of days for each mouse to reach the 70% success criterion in S trials, (H(2) = 6.29, p=0.043; Kruskal Wallis)(Cre+ DCZ comparison to Cre+ DMSO, p = 0.017; Cre+ DCZ comparison to Cre- DCZ, p = 0.05 with Dunn's multiple comparison post hoc analysis).
(E) c-Fos+ cell density in all groups of mice. There was an increase in c-Fos+ mGCs in mice in which ≤ 7 week abDGCs were inhibited by DCZ (H(2) = 7.22, p=0.0271; Kruskal Wallis)(Cre+ DCZ comparison to Cre+ DMSO, p = 0.014; Cre+ DCZ comparison to Cre- DCZ, p = 0.027 with Dunn's multiple comparison post hoc analysis).
(F) Example section after c-Fos immunohistochemistry to assess distribution of c-Fos+ mGCs between the IB and SB of the dorsal DG in groups of mice who received DCZ (cre- vs cre+). c-Fos cells are localized closer to the hilus in the Cre+ group. Calibration bar, 100 µm.
(G) c-Fos+ density in the CA3 region of the hippocampus in each of the groups of mice. No significant difference was observed in any of the groups (H(2) = 3.9, p =0.142; Kruskal Wallis).
(H) Blade distribution of c-Fos+ mGCs in the IB and SB of the dorsal DG in mice performing the LS separation receiving either DCZ or DMSO 30 min prior to task performance. Active neurons are distributed preferentially to the SB than the IB in the DG for the control groups. However this distribution bias is significantly reduced in Cre+ DCZ group (IB: H(2) = 8.91, p = 0.012; SB: H(2) = 8.910, p = 0.012; Kruskal Wallis)(Cre+ DCZ comparison to Cre- DCZ p = 0.0032; Cre+ DCZ comparison to Cre+ DMSO p = 0.036 with Dunn's multiple comparison post hoc analysis).

(I) Spatial distribution of c-Fos+ GCs along hilar to molecular layer axes of the SB (0 to 120µm) and the IB (0 to 120µm) of the dorsal DG, with 0 µm indicating the hilar position. c-Fos+ cells are localized closer to the hilus in the Cre+ DCZ group.
(J) Cartoon illustrating the DG with its two blades: IB and SB. Dentate granule cells are depicted in grey while red circles represent activity labelled cells in the DG. Distribution of labelled neurons is closer to hilus, away from outer radial GCL and more evenly distributed in IB and SB.

Figure 5: **DREADD inhibition of ≤ 4-week-old abDGCs does not affect performance of mice**
(A) Schematic representation of the pattern separation paradigm used in the study. Prior to the behavioral experiment, mice (males n = 12, females n = 23) were given tamoxifen-enriched food for 4 weeks to activate the Cre recombinase and label newborn neurons. All mice received DCZ or DMSO injections 30 minutes before the beginning of LS trials.
(B) Days for each mouse to reach the 70% success criterion in S trials (H(2) = 4.21, ns; Kruskal Wallis).
(C) Density of c-Fos+ GCs in the dorsal DG across the different groups (DG: H(2) = 0.404, ns, Kruskal Wallis)
(D) Blade specific distribution of c-Fos+ cells in the IB and SB of the dorsal DG in mice performing LS separation (H(2) = 0.46, ns ; Kruskal Wallis).
(E) Spatial distribution of c-Fos+ along the hilar to outer radial GCL axis of the DG (0 to 120µm), with 0 µm indicating location at the hilus.

Figure 6: **DREADD inhibition of mGCs during a high cognitive demand task**
(A) Schematic representation of the pattern separation paradigm. All mice (males n = 17, females n = 27) received DCZ i.p. 30 minutes before the beginning of their LS trials.
(B) Percentage of mice reaching criterion in (S) and percentage that were not successful (NS) after 14 days of training in S configuration of LS. A majority of mice never succeeded in reach the criterion in the Cre+ DCZ group (z = 2.00, p = 0.0453, z-test - Group NS).
(C) Percentage of mice reaching the criterion in S trials across days represented as the cumulative probability including all successful and not successful mice in the group.
(D) Density of c-Fos+ GCs in the dorsal DG across the two groups. A significantly reduced density was observed in the Cre+ DCZ group compared to controls (DG, Cre+ DCZ: 8933.9 ± 556.4 mm^3, 6 mice; Cre- DCZ: 13578 ± 2135 mm^3, 5 mice; p = 0.045; Mann–Whitney).

(E) Density of c-Fos+ GCs in the dorsal CA3 across the two groups. A significant reduction was observed in the Cre+ DCZ group compared to the control groups (CA3 Cre+ DCZ: 20647 ± 1309 mm^3, 6 mice; Cre-DCZ: 34500 ± 2665 mm^3, 5 mice; p= 0.0062; Mann–Whitney).
(F) Blade specific distribution of c-Fos+ GCs in the IB and SB of the dorsal DG in mice performing LS separation (IB: Cre- DCZ: 31.5 ± 2.47 %, 5 mice; Cre+ DCZ: 27.2 ± 2.55 %, 6 mice; ns; SB: Cre- DCZ: 68.6 ± 2.47 %, 5 mice; Cre+ DCZ: 72.8 ± 2.55 %, 6 mice; ns; Mann–Whitney).
(G) Spatial distribution of c-Fos+ along the hilar to outer radial GCL axis of the DG (0 to 120µm), with 0 µm indicating location at the hilus.
(H) Cartoon illustrating the distribution of activity labeled GCs in the SB and IB blades of the DG. GCs are depicted in grey while red circles represent activated cells in the DG. The overall distribution of neurons in mice in which mGCs were inhibited (Cre+ DCZ) and which did not reach criterion was not different to controls even though the total density of activity labelled neurons was lower.

Figure Sup1: **Activity labeling of GCs after running**
(A) Schematic representation of the running paradigm. Prior to the behavioral experiment, mice (males n = 30, females n = 3) were injected with BrdU. After Δ weeks, the mice were allowed to run for 2 hours before receiving a 4-OHT injection, followed by an additional 2 hours of running. Mice were perfused 3 days later for immunohistochemistry of brain sections.
(B) Example section of immunohistochemistry of TRAP+ cells in the dorsal DG illustrating the distinct blades of the DG: SB and IB. Calibration bar: 40 µm.
(C) Density of TRAP+ GCs in mice after running compared to home cage controls (H). Each group represents mice that ran after variable time intervals following BrdU injections.
(D) Blade specific distribution of TRAP+ cells in the infrapyramidal (IB) and suprapyramidal (SB) blades of the dorsal DG in TRAP2 mice that ran for 4 hours, compared to home cage mice. (IB: home: 42.5 ± 1.43 %, 4 mice; run: 43.9 ± 1.42 %, 33 mice; ns; Mann–Whitney).
(E) Example section from mice labelled for BrdU (green), TRAP+ GCs (red) and endogenous c-Fos (blue) in TRAP2 mice. Calibration bar: 20 µm.
(F) Percentage of BrdU cells (Δ 1-14 weeks old) also labelled with TRAP in the dorsal DG of TRAP2 mice that ran for 4 hours, compared to home cage controls (H).

Figure Sup 2: **Description of the TUNL protocol and analysis of TRAP+ GCs in mice not meeting the criterion for success in the pattern separation task.**

(A) Overview of successive trials in the Shaping, L and LS steps. Mice must complete 30 trials at each step. The shaping phase involves touching the illuminated square to receive a reward. In the L and LS steps, there are in two phases: the sample (S) phase, where mice must touch the single illuminated square on the panel and the choice test (C), where two squares are illuminated: the previously touched square and a new one. Mice must nose poke the new square to receive the reward. In the L phase, trials consist only of L panels only (see sup2B – L). In the LS phase, L and S trials are alternated (see sup2B for S panels).
(B**)** Recommended stimulus pairs for the TUNL protocol. Large pairs (green) consist of 2 illuminated squares separated by 4 squares that are turned OFF. Small pairs (orange) consist of two illuminated squares separated by 0 to 1 square turned OFF.
(C) Percentage of TRAP2 mice reaching the criterion (S) compared to those that did not within 14 days of training (NS).
(D) Density of TRAP+ neurons in mice undergoing LS for mice that reached criterion (LS S) with mice that did not reach 70% criterion within 14 days (LS NS) (LS S: 2563 $\pm$ 481 mm^3, 8 mice; LS NS: 2036 $\pm$ 305 mm^3, 10 mice; ns; Mann–Whitney).
(E) Blade specific distribution of TRAP+ GCs in the SB and the IB of the dorsal DG (IB: LS S: 34 $\pm$ 2.79 %, 8 mice; LS NS : 33.8 $\pm$ 2.12 %, 12 mice; ns; Mann–Whitney).
(F) Percentage of TRAP+ cells also birth-dated with BrdU (39 days +/- 1.23) or Edu cells (25 days +/- 1.37 days) in the dorsal DG of TRAP2 mice that reached criterion (S) and those failed (NS)(BrdU: LS S: 0.71 $\pm$ 0.333 %, 7 mice; LS NS : 0.822 $\pm$ 0.45 %, 6 mice; ns; EdU: LS S: 0.542 $\pm$ 0.33 %, 9 mice; LS NS : 0.304 $\pm$ 0.16 %, 7 mice; ns; Mann–Whitney).

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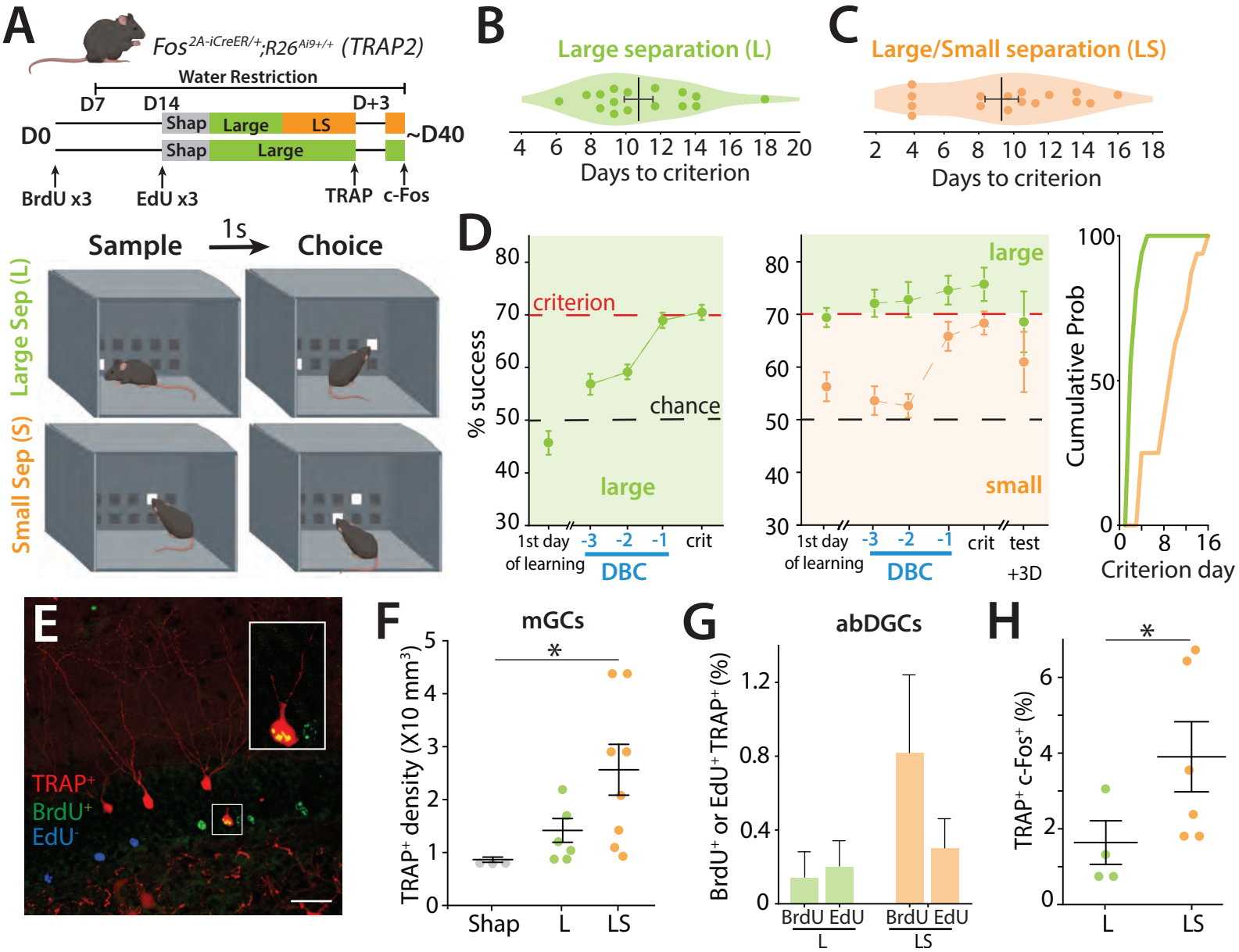

A
Fos2A-iCreER/+;R26Ai9+/+ (TRAP2)
Water Restriction
D7
D14
D+3
D0
Shap
Large
LS
~D40
BrdU x3
EdU x3
TRAP
c-Fos
Sample
1s
Choice
Large Sep (L)
Small Sep (S)
B
Large separation (L)
Days to criterion
C
Large/Small separation (LS)
Days to criterion
D
% success
criterion
chance
large
small
1st day of learning
DBC
crit
test
+3D
Cumulative Prob
Criterion day
E
TRAP+
BrdU+
EdU-
F
mGCs
TRAP+ density (X10 mm3)
Shap
L
LS
G
abDGCs
BrdU+ or EdU+ TRAP+ (%)
BrdU EdU
H
TRAP+ c-Fos+ (%)

Figure 1

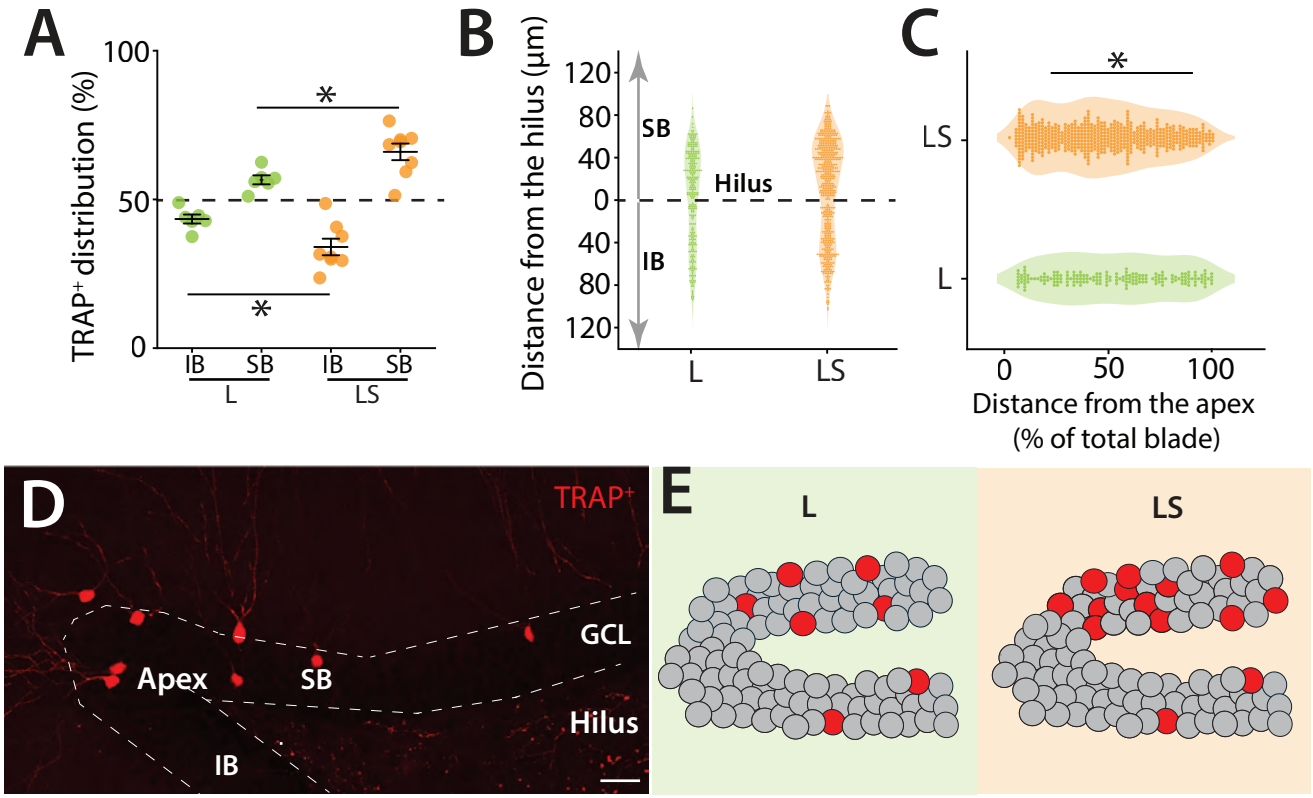

A
100
50
0
TRAP+ distribution (%)
*
*
IB
SB
IB
SB
L
LS
B
Distance from the hilus (µm)
120
80
40
0
40
80
120
SB
IB
Hilus
L
LS
C
*
LS
L
0
50
100
Distance from the apex
(% of total blade)
D
TRAP+
GCL
Apex
SB
Hilus
IB
E
L
LS

Figure 2

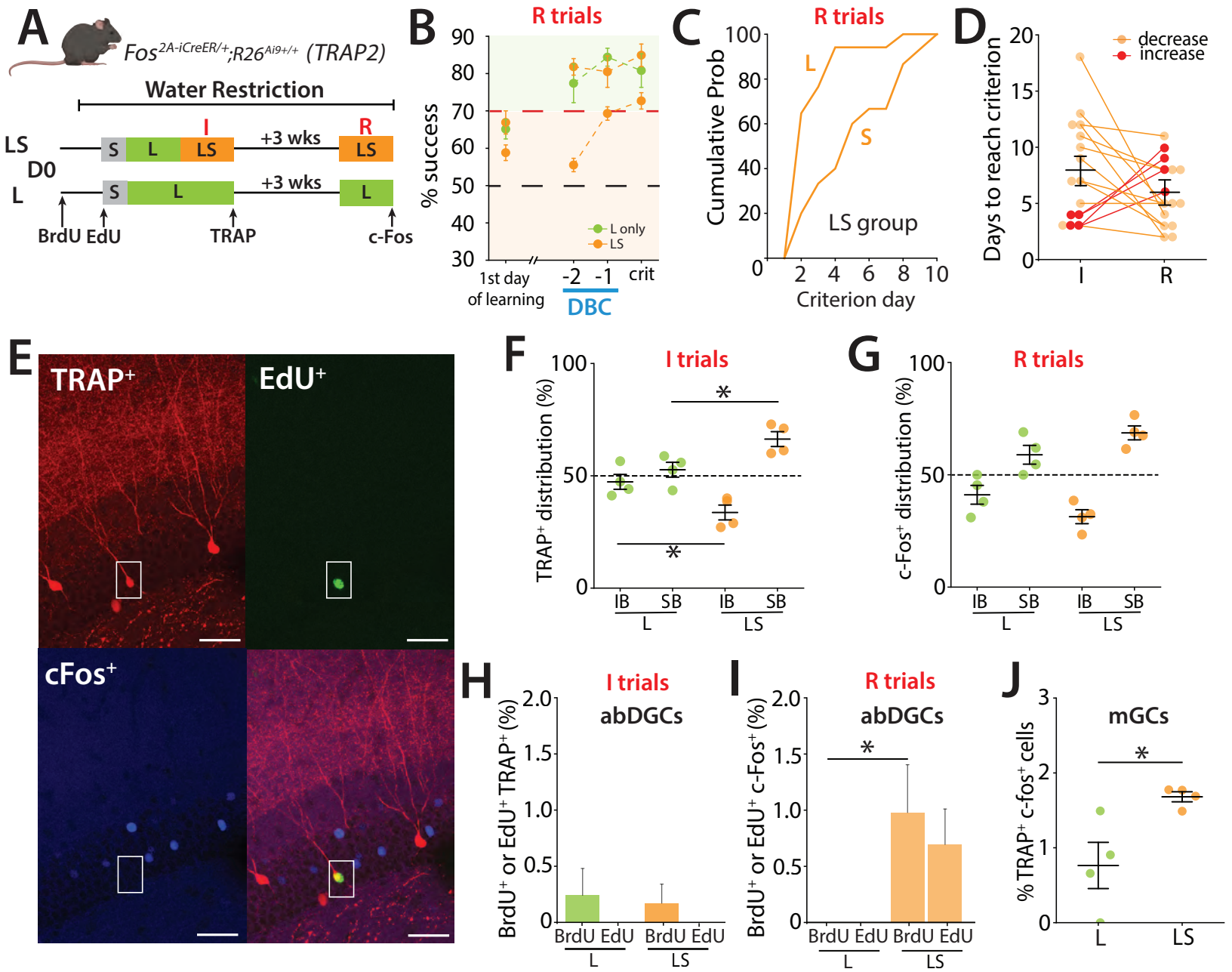

A
Fos2A-iCreER/+;R26Ai9+/+ (TRAP2)
Water Restriction
LS
D0
L
S
L
LS
I
+3 wks
R
LS
S
L
+3 wks
L
BrdU EdU
TRAP
c-Fos
B
R trials
% success
L only
LS
1st day of learning
-2
-1
crit
DBC
C
R trials
Cumulative Prob
L
S
LS group
Criterion day
D
decrease
increase
Days to reach criterion
I
R
E
TRAP+
EdU+
cFos+
F
I trials
TRAP+ distribution (%)
IB
SB
IB
SB
L
LS
G
R trials
c-Fos+ distribution (%)
IB
SB
IB
SB
L
LS
H
I trials
abDGCs
BrdU+ or EdU+ TRAP+ (%)
BrdU EdU
BrdU EdU
L
LS
I
R trials
abDGCs
BrdU+ or EdU+ c-Fos+ (%)
BrdU EdU
BrdU EdU
L
LS
J
mGCs
% TRAP+ c-fos+ cells
L
LS

Figure 3

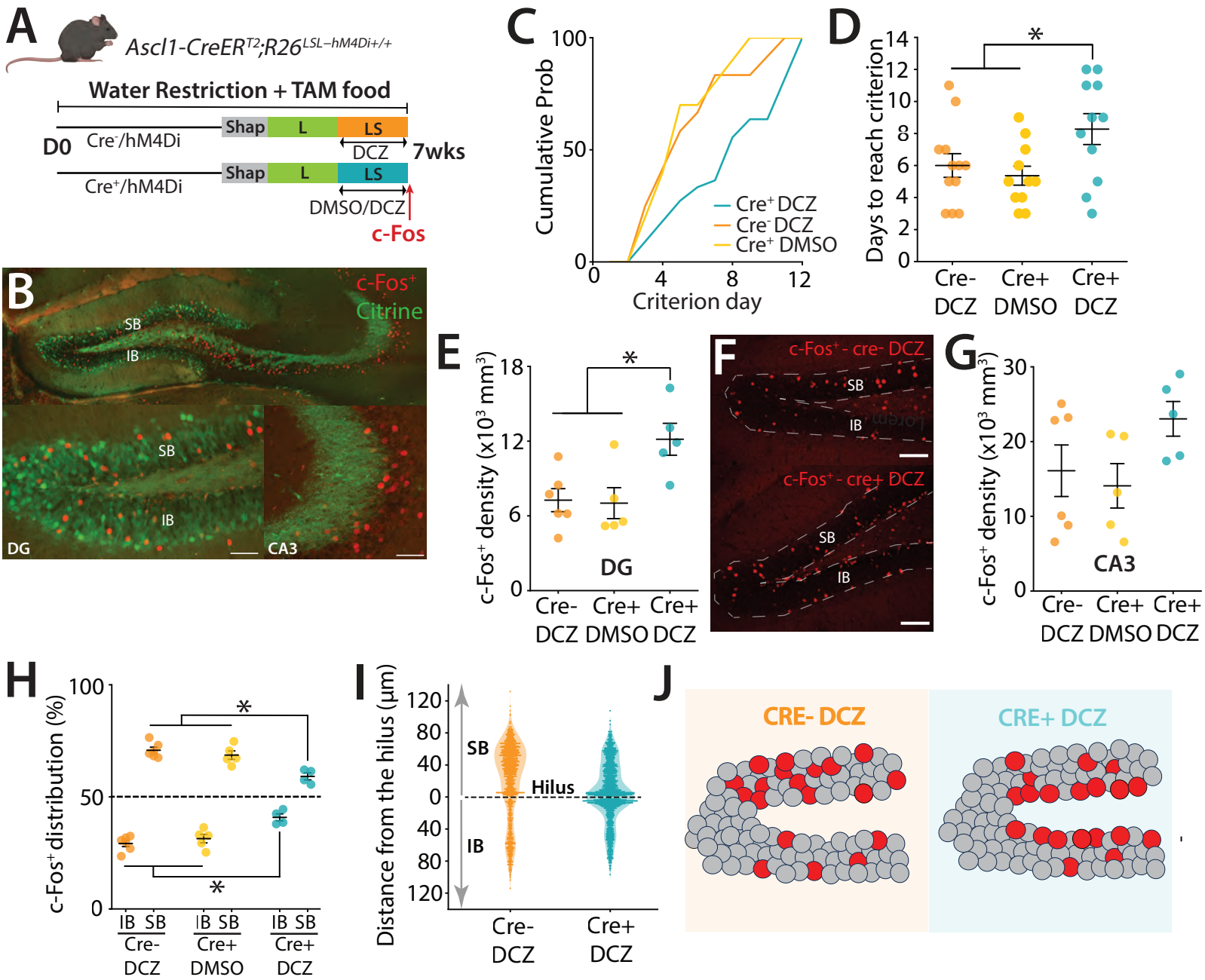

A
Ascl1-CreER^{T2};R26$^{LSL-hM4Di+/+}$
Water Restriction + TAM food
D0
Cre$^-$/hM4Di
Shap
L
LS
DCZ
7wks
Cre$^+$/hM4Di
DMSO/DCZ
c-Fos
B
c-Fos$^+$
Citrine
SB
IB
DG
CA3
C
Cumulative Prob
Criterion day
Cre$^+$ DCZ
Cre$^-$ DCZ
Cre$^+$ DMSO
D
Days to reach criterion
Cre-
DCZ
Cre+
DMSO
Cre+
DCZ
E
c-Fos$^+$ density (x10^3 mm^3)
DG
F
c-Fos$^+$ - cre- DCZ
c-Fos$^+$ - cre+ DCZ
G
CA3
H
c-Fos$^+$ distribution (%)
IB SB
I
Distance from the hilus (µm)
Hilus
J
CRE- DCZ
CRE+ DCZ

Figure 4

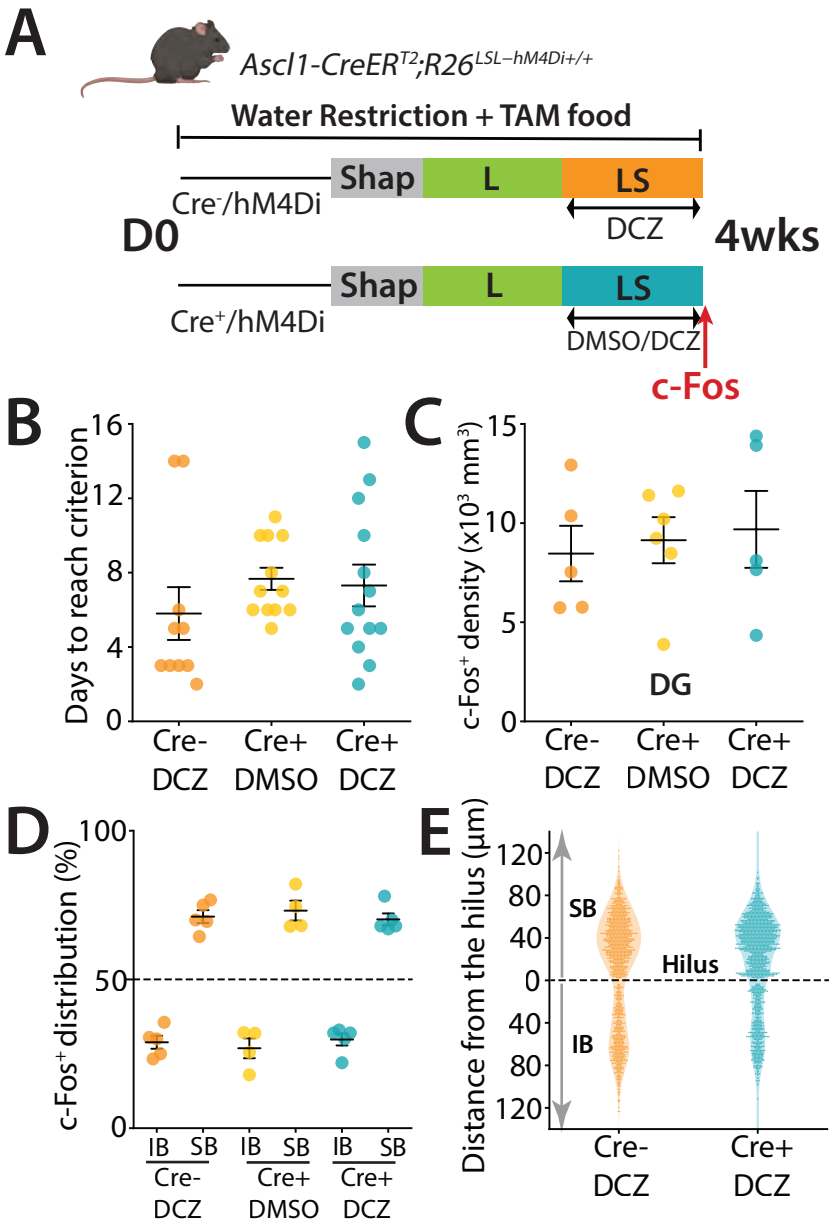

A
Ascl1-CreER^{T2};R26$^{LSL-hM4Di+/+}$
Water Restriction + TAM food
Shap
L
LS
Cre^{-}/hM4Di
D0
DCZ
4wks
Shap
L
LS
Cre^{+}/hM4Di
DMSO/DCZ
c-Fos
B
Days to reach criterion
16
12
8
4
0
Cre-
DCZ
Cre+
DMSO
Cre+
DCZ
C
c-Fos^{+} density (x10^{3} mm^{3})
15
10
5
0
DG
Cre-
DCZ
Cre+
DMSO
Cre+
DCZ
D
c-Fos^{+} distribution (%)
100
50
0
IB
SB
IB
SB
IB
SB
Cre-
DCZ
Cre+
DMSO
Cre+
DCZ
E
Distance from the hilus (µm)
120
80
40
0
40
80
120
SB
Hilus
IB
Cre-
DCZ
Cre+
DCZ

Figure 5

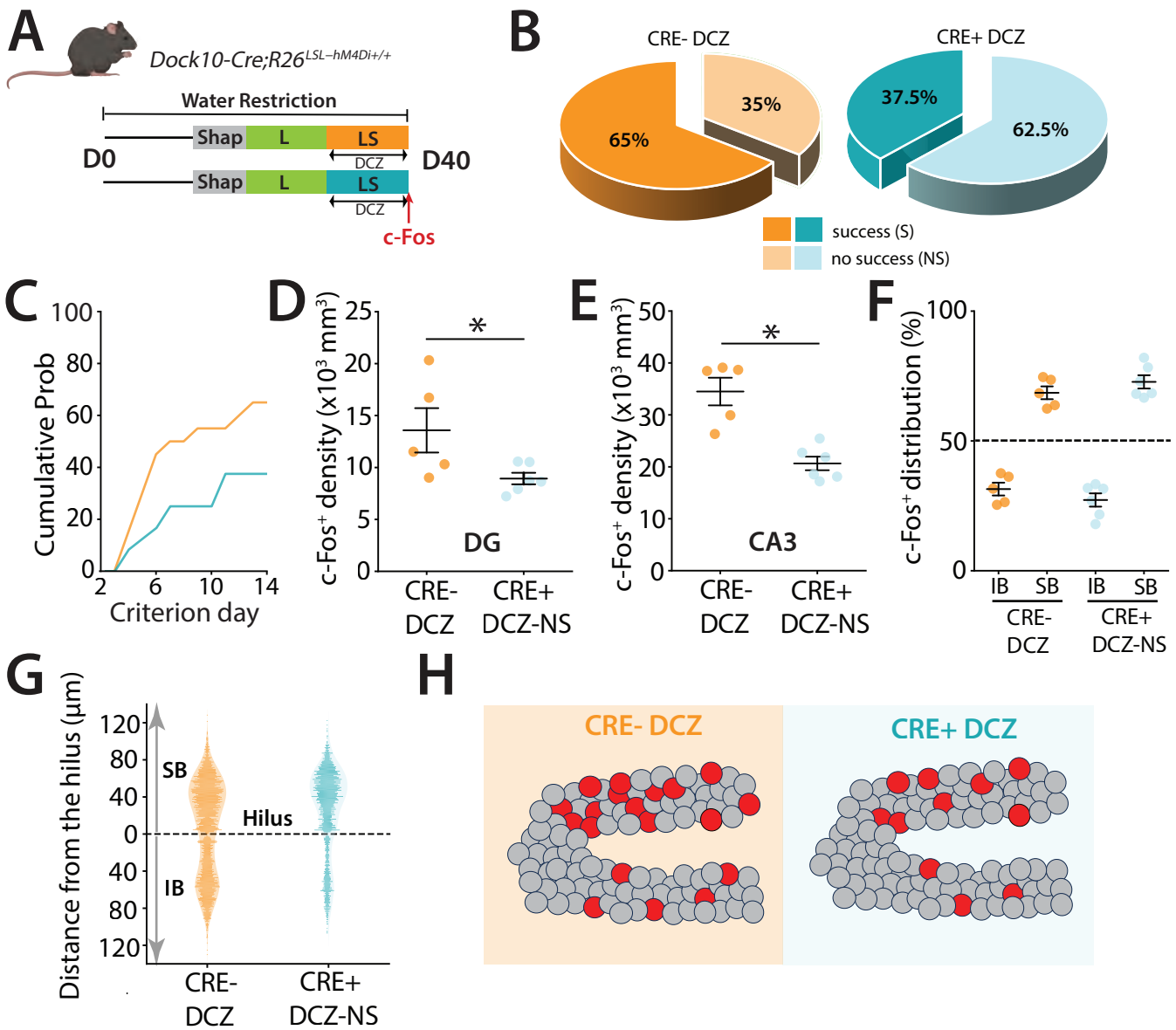
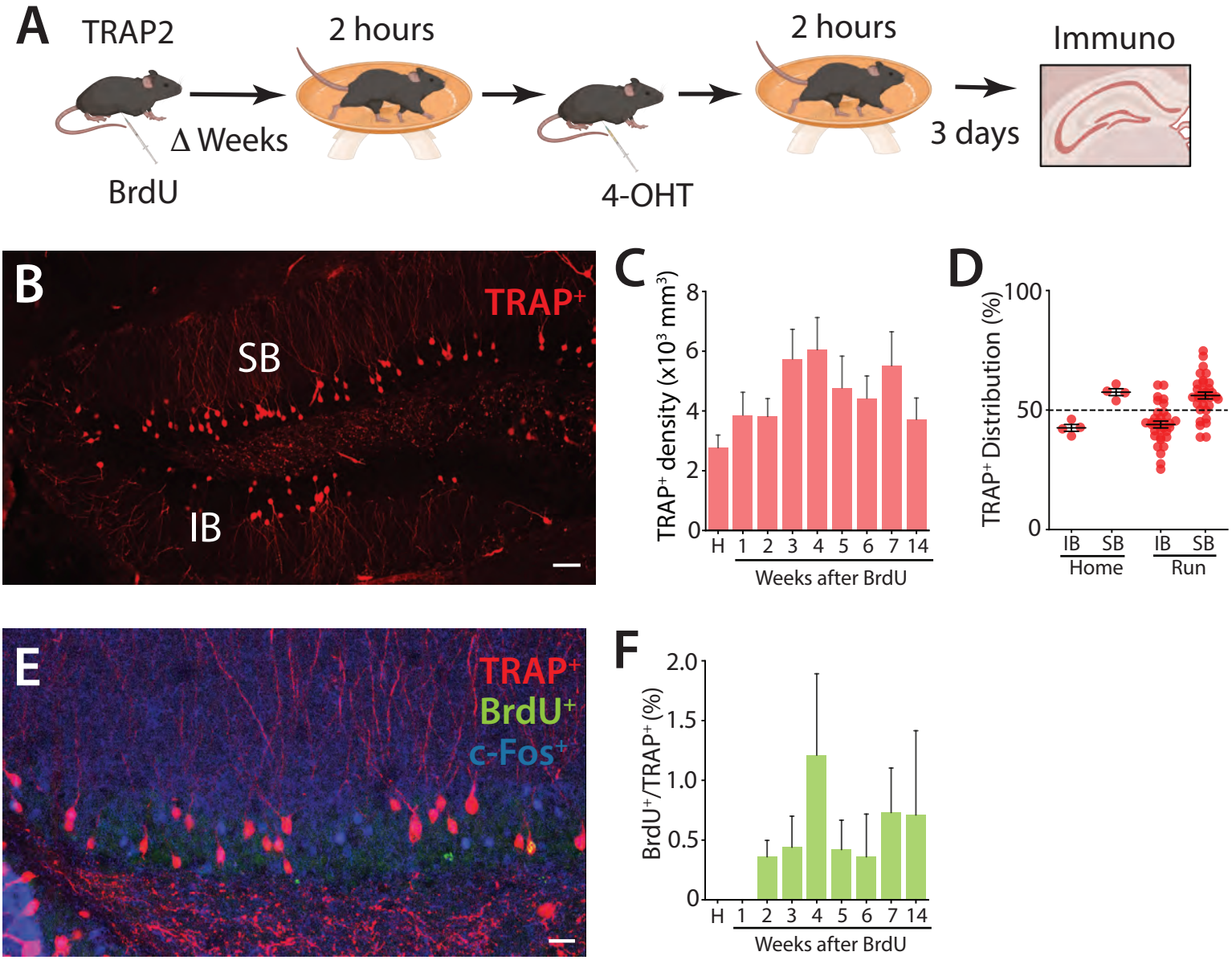
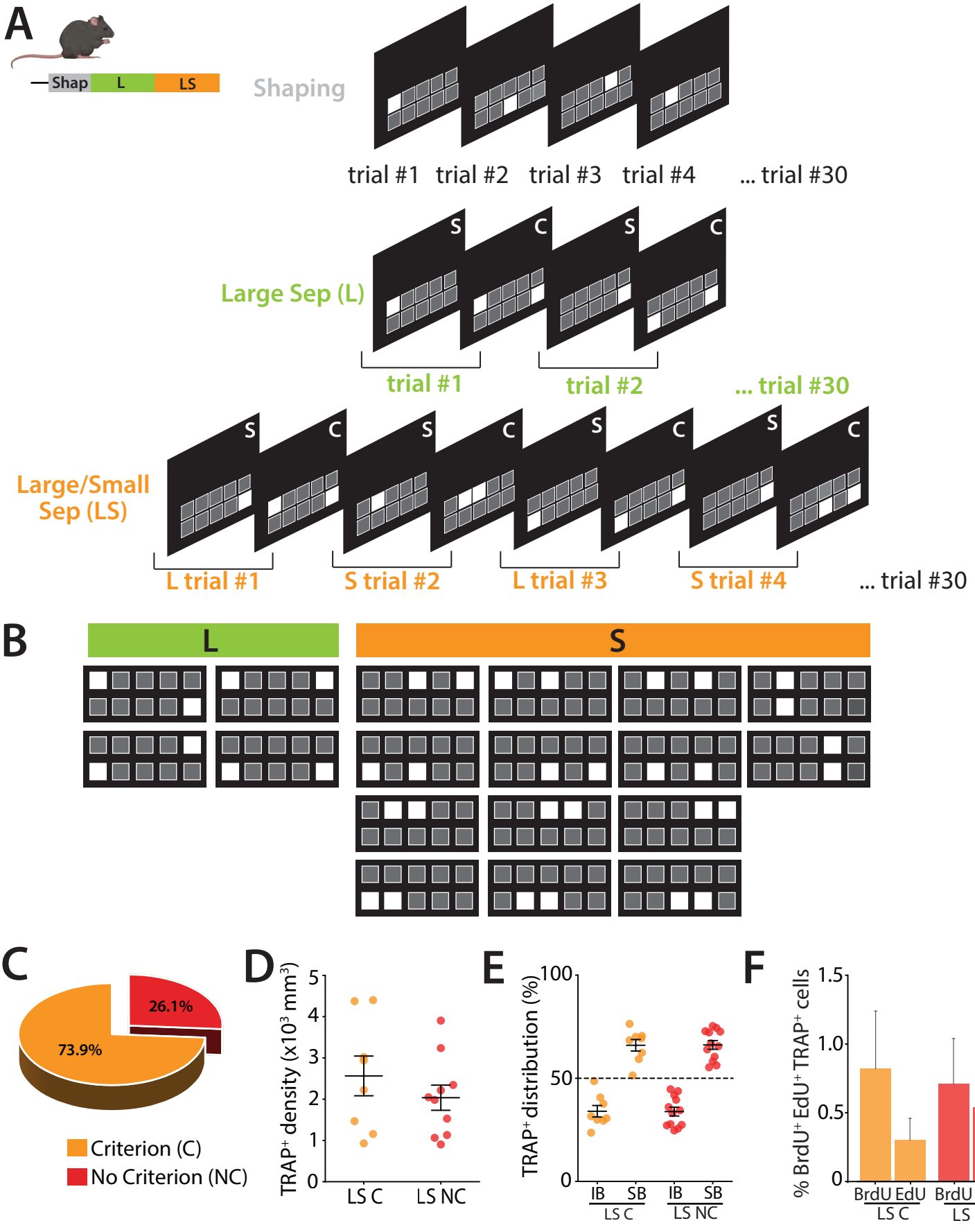

A
Dock10-Cre;R26LSL–hM4Di+/+
Water Restriction
Shap
L
LS
D0
D40
DCZ
c-Fos
B
CRE- DCZ
CRE+ DCZ
65%
35%
37.5%
62.5%
success (S)
no success (NS)
C
Cumulative Prob
Criterion day
D
c-Fos+ density (x10³ mm³)
DG
CRE-
DCZ
CRE+
DCZ-NS
E
CA3
F
c-Fos+ distribution (%)
IB
SB
G
Distance from the hilus (µm)
Hilus
H
CRE- DCZ
CRE+ DCZ

Figure 6



Suppl Figure S1



Suppl Figure S2