# Synaptic plasticity, place cells and spatial memory: study with second generation knockouts

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**The use of genetically engineered mice has been a major development in neuroscience research. Genetic engineering is an undoubtedly powerful technique; however, the value of this approach has been debated, particularly in relation to its use to probe the underlying bases of complex behaviors such as memory. A recent new development of the technique is the ability to target a specific gene knockout to a particular subregion or even to specific and limited cell types of the mouse brain. An example of this approach is the knockout of the *NMDAR1* gene in only CA1-pyramidal cells of the hippocampus. The resulting animals can be tested by several methods, including *in vivo* multielectrode recording during behavioral tasks. The data provide strong evidence in favor of the notion that NMDA receptor-dependent synaptic plasticity at CA1 synapses is required for both the acquisition of spatial memory and the formation of normal CA1 place fields. This relationship suggests that robust place fields may be essential for spatial memory.**

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GENETIC MUTANTS have been a powerful tool in deciphering molecular and cellular mechanisms underlying a variety of complex biological processes such as development[1]. In neuroscience, the application of this approach has been limited to organisms with relatively simple nervous systems such as *Drosophila*[2,3]. This situation was altered drastically with the introduction of the mouse gene-knockout technology[4]. Since Alcino Silva and his associates published their results on α-$Ca^{2+}$–calmodulin kinase type II (αCaMKII) knockout mice in 1992 (Refs 5,6), more than 50 papers have appeared that utilize knockout mice to address the relationship between the function of a specific gene and synaptic plasticity or behavior[7].

Can knockout mice be as powerful in the analysis of behavior and cognition as fly mutants have been in the dissection of, for example, development? Or is the complexity of the mammalian CNS so great that even the powerful genetic approach will succumb to it? Recently, this and related issues have been debated in several publications[8–10] including *TINS* (Refs 11,12). Our view is that the genetically engineered mice will be enormously useful in neuroscience. However, some obvious drawbacks accompanying the conventional knockout technology have to be resolved before this approach becomes truly effective. The major drawback of the current gene-knockout technology, as applied to the brain, is the lack of regional and temporal specificity. Mammalian brains consist of highly organized, interconnected subregions, each performing distinct functions. Many of these functionally distinct brain subregions express a common set of genes, such as those for neurotransmitter receptors and enzymes in signal transduction. When one of these genes is knocked out by the conventional method, it is absent throughout the brain, and in fact throughout the entire organism. It is therefore, often difficult or impossible to attribute an impairment observed at the behavioral or electrophysiological level to the lack of the normal gene in a particular subregion of the brain. An additional uncertainty arises because many genes play a role not only in the functioning of a developmentally mature brain but also during the development. The observed behavioral and other defects could have arisen indirectly from the animal's inability to execute its normal developmental program.

## Selective gene-knockout technology

By combining the phage P1-derived, *Cre–lox* recombination system[13] with the embryonic stem cell gene-targeting technology, we have recently succeeded in developing a second generation of knockout mouse technology, in which deletion of a specific gene can be restricted to one particular type of cell in the



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brain (Fig. 1A)[14]. In particular, we created a *Cre* transgenic mouse line (T29-1) in which a DNA sequence flanked by a pair of *loxP* sequences is deleted efficiently (>97%) and exclusively in the CA1 pyramidal cells of the hippocampus (Fig. 1B). An additional advantage of this CA1-restricted knockout system is that the *Cre–loxP*-mediated deletional recombination does not occur until the third postnatal week[14], by which time cellular organization and connections have been established in the CA1 region. Thus, CA1 knockout mice display not only regional but also temporal restriction of the gene deletion, reducing developmental concerns. We crossed the T29-1 mice with a second mouse line in which *loxP* sequences were targeted into the gene encoding the $NMDA_1$ receptor [dubbed *floxed NR1* (*fNR1*) gene][15]. The *in situ* hybridization data demonstrated that the intact gene encoding the $NMDA_1$ receptor is missing specifically in the CA1 region in the *Cre*-transgenic *fNR1* homozygous mice (CA1-KO mice). These mice grow apparently normally and are fertile in a stark contrast to the perinatally lethal $NMDA_1$-receptor knockout mice produced previously by the conventional knockout technology[16].

### Abnormalities in synaptic plasticity and spatial memory

Using whole-cell patch clamp and field recording techniques we found that the CA1-KO mice lack NMDA receptor-mediated excitatory postsynaptic currents (EPSC) or potentials (EPSP) at the Schaffer collateral–CA1 synapses, but the EPSC mediated by the AMPA receptor is normal. By contrast, as expected from the CA1 specificity of the deletion, NMDA receptor-mediated EPSPs at perforant path–dentate gyrus synapses are completely normal. We also found that application of tetanic stimulation (100 Hz for 1 s) failed to induce long-term potentiation (LTP) at the Schaffer collateral–CA1 synapses in the CA1-KO slices (Fig. 2A). Short-term potentiation (STP) and long-term depression (LTD) (normally induced by 1 Hz for 10 min) were also deficient at the CA1 synapses. By contrast, LTP at the perforant pathway synapses in the dentate gyrus (40 shocks at 100 Hz) was indistinguishable from that of control mice (Fig. 2B).

We then subjected the CA1-KO mice to Morris water-maze tasks in order to test the hypothesis that hippocampal synaptic plasticity and, more specifically, the plasticity involving CA1 pyramidal cells are essential for the formation of spatial memory, a form of declarative memory. We found that in contrast to three types of control littermates, namely wild-type mice, T29-1 transgenic (but without the *fNR1* alleles) mice and *fNR1* homozygous (but without the *Cre* transgene), CA1-KO mice are severely deficient in the acquisition of spatial memory (learning of the position of the hidden platform by using the relationships among distal cues around the pool). This was demonstrated by showing that the CA1-KO mice, in contrast to control mice, do not swim preferentially in the target quadrant in the 'transfer test' (Fig. 2C). The impaired behavior of the mutant mice in the hidden-platform task was not due to some sensorimotor or motivational deficit because the CA1-KO mice could reach the same level of optimal performance as the control mice in a simpler task in which the position of the hidden platform was marked by proximal landmark.

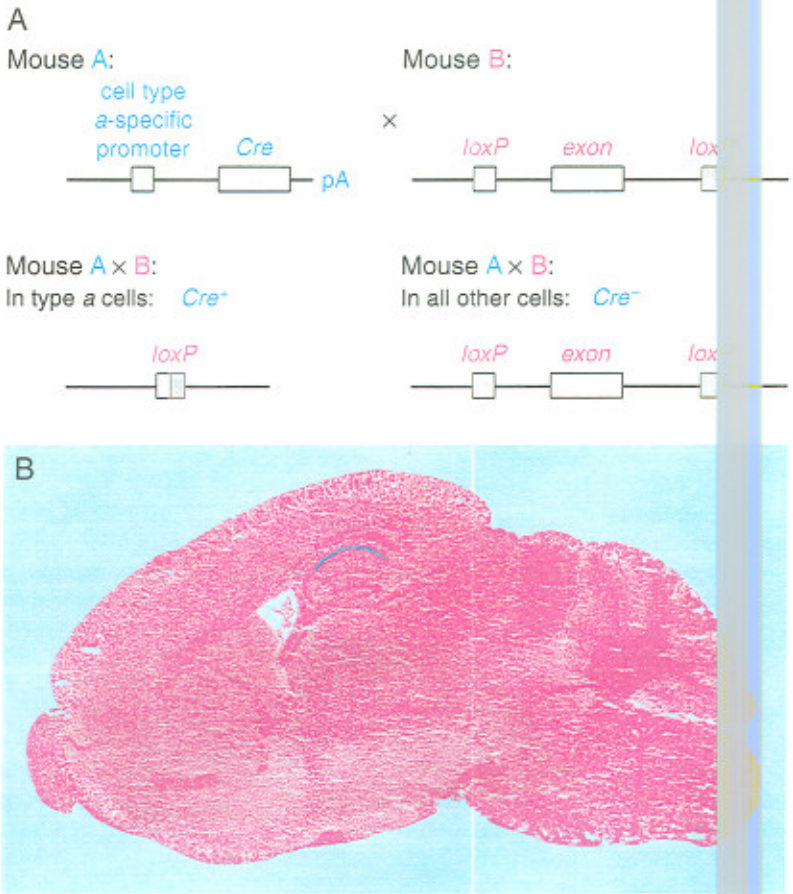


**Fig. 1.** *The* Cre–loxP *system for cell type-restricted gene knockout.* **(A)** *Strategy for c ype-restricted gene knockout.* **(B)** *CA1 pyramidal cell-restricted* Cre–loxP *recombination. A ouse line transgenic for the* Cre *recombinase gene under the control of α-*$Ca^{2+}$*–calmoduli ase type II (αCaMKII) promoter was crossed to a* lacZ *reporter transgenic mouse in w the expression of β-galactosidase depends on* Cre–loxP*-mediated recombination (E. Mer and D. Anderson, unpublished observations). The sagittal brain section derived from a uble transgenic mouse was stained with X-Gal and then counterstained with eosin. The arc depicts the X-gal-stained CA1 pyramidal-cell layer.*

### CA1 place cells

The combined electrophysiological and behavioral studies on the CA1-KO mice provide strong evidence for the notion that synaptic plasticity at CA1 synapses is essential for spatial learning. The next major question is how the lack of CA1 synaptic plasticity disrupts spatial learning. In order to address this issue we examined the activity of CA1 place cells. Place cells were discovered by *in vivo* hippocampal electrical recordings as pyramidal cells that fire at a heightened rate in a site-specific manner as a rodent moves freely throughout its environment[17]. Each cell has its own region of elevated firing, termed a place field, and large numbers of hippocampal cells will fill each environment with overlapping place fields. Information about the location of an animal is of high-enough quality that the position can be estimated well by simultaneously examining firing patterns of many hippocampal neurons[18]. The relative location of these place receptive fields changes in different environments, and thus, place fields clearly must be learned anew in each environment with spatial information represented in the firing of ensembles rather than single cells.

The use of multiple-electrode recording techniques in CA1-KO mice that are able to explore freely a defined space provides an ideal opportunity to examine

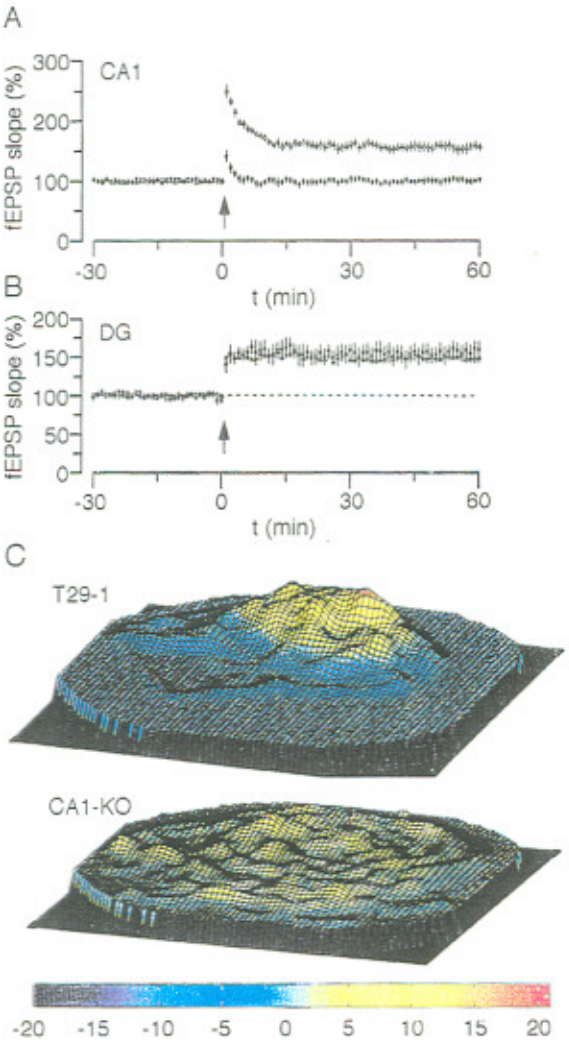


Fig. 2. In vitro *electrophysiology and behavior in NMDA$_1$-receptor CA1 knockout (CA1-KO) and control mice.* (A) *Lack of CA1 long-term potentiation (LTP) in CA1-KO mice. The mean (±SEM) field excitatory postsynaptic potentials (fEPSPs) in two groups of mice tested for LTP induction at Schaffer collateral–CA1 synapses. CA1-KO (closed circles, n = 21) did not show LTP, whereas the other group presented clear LTP (fNR1, upward triangles; n = 4).* (B) *The mean (±SEM) fEPSPs in the CA1-KO (closed circles; n = 10) and fNR1 (open triangles; n = 6). Significant LTP was elicited in both groups after the tetanus.* (C) *Three-dimensional graphs representing the total occupancy of six T29-1 mice and six CA1-KO mice during the transfer test in the Morris water maze. The control mice focused their search in the trained location whereas the mutant mice visited the whole maze area equivalently.*

the relationship between synaptic plasticity, place-cell formation and spatial memory[19]. Mice were fitted with microdrive arrays containing six independently adjustable tetrodes. The general format of the experiments consisted of multiple behavioral sessions within a linear track, an L-shaped track or an open field, bracketed by sleep periods which allow recording stability to be assessed – a necessary condition for evaluating true changes in firing characteristics. We measured a variety of basic characteristics of both excitatory and inhibitory hippocampal CA1 neurons in the CA1-KO mice and control littermates, such as overall firing rates and basic spike waveform, and found that the basic neuronal firing characteristics are normal in knockout mice during behavior. Despite having no NMDA receptor-dependent plasticity, CA1 pyramidal cells in knockout mice showed a surprising degree of place-related firing. Preliminary evaluation of a small number of cells in area CA3, which provides a major input to CA1, also did not reveal any significant disturbance of place-related activity in the CA1-KO mice. By examining the spatial pattern of firing of CA1 pyramidal cells over repeated exposures to the same environment we found that cells exhibit stable place fields for at least one hour. This indicates that these mice are not remapping the environment upon every exposure, and suggests that they recognize the environment from previous experience.

## Abnormalities in CA1 place cells

Interestingly, CA1 pyramidal cells from CA1-KO mice displayed two major abnormalities compared to the cells from control mice. The first abnormality was that while place-related firing of CA1 pyramidal cells is preserved substantially in the CA1-KO mice, spatial specificity of place fields is clearly poorer than in control littermates (Fig. 3A). Place fields recorded from control animals usually have a single salient peak while place fields recorded from knockout mice, while sometimes individually indistinguishable from control fields, were more likely to be broad and diffuse, with multiple peaks. The combined data from the linear and L-shaped tracks indicated that the mean values of the size of the place field of the CA1-KO mice were greater by about one third than those of the control mice (Fig. 3B). How might the deletion of the gene encoding the NMDA$_1$ receptor in the CA1 region lead to reduced specificity of place fields among CA1 neurons? To address this issue we constructed a model of the random hardwired connections of the CA3 network to a single postsynaptic CA1 cell, similar in architecture to that which exists in the CA1-KO mice. The numbers of neurons and connections for these brain regions were matched to values reported in the literature, and we assumed that the place fields are normal in CA3. We also added a firing threshold that adapts to average or peak postsynaptic activity. Without plasticity, but with an adapting firing rate, we obtain location-dependent firing in CA1 cells with poor specificity, as we found in CA1-KO mice. What is gained with plasticity? Using a conventional 'Hebbian' learning rule, the model produced a more specific place field transmitted from CA3 to CA1 compared to the case without plasticity.

Is the one-third increase in place-field size of the individual CA1 neurons sufficient to explain the severe deficiency in spatial learning that is observed in the CA1-KO mice? In fact, the observation that 30–50% of cells within the hippocampus become active within a given environment indicates strongly that rodents use ensemble representations of location rather than mapping places to individual cells. In order for an ensemble code to provide accurate spatial information there must be robust co-variance of the firing of cells that have overlapping place fields. We took advantage of our multiple electrode array to measure the degree of co-ordinated firing between neurons that have overlapping place fields (co-variance coefficient) and uncovered a second major abnormality in the mutant CA1 cells: pairs of cells of CA1-KO mice exhibit completely uncorrelated firing while control pairs exhibit significant correlations (Fig. 4A). This dramatic effect in CA1-KO mice means that

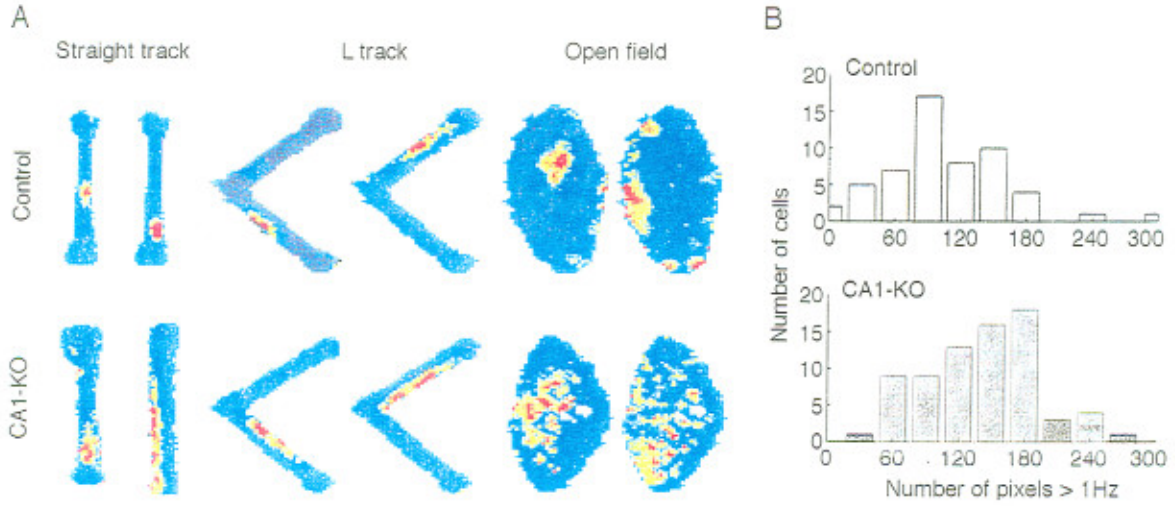


Fig. 3. *Place fields of NMDA$_1$-receptor CA1 knockout (CA1-KO) mice are significantly larger in all behavioral environments.* (A) *Rate maps of place-specific activity of two pyramidal cells from control animals and two pyramidal cells from knockout animals in each behavioral environment. The peak rate of each panel has been adjusted to reveal areas of highest activity. The field sizes of the pyramidal cells of the CA1-KO animals were significantly larger in both the linear track (one-dimensional) environment and the two-dimensional open-field environment.* (B) *Histogram demonstrating the distribution of CA1 pyramidal-cell field sizes in control (n = 55 cells) and mutant animals (n = 74 cells). The mean field size in CA1-KO animals was 140.3 pixels (~560 cm$^2$) while in control animals the mean size was 106.0 pixels (~420 cm$^2$).*

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downstream regions cannot use these correlations to learn about the space.

This point is elaborated by examining the estimate of the location of an animal conveyed simultaneously by multiple cells – the ensemble representation of location. We were able to record from as many as 29 neurons simultaneously, and were thus able to estimate the position of the animal from knowledge of their fields and spike trains alone. For small numbers of cells, the ensemble of neurons in knockout mice carry as much information about location as the ensemble in controls. However, this information is rather poor and results in large errors in the estimate of the location of the mouse. As the number of cells included in the analysis increased, their place fields overlapped more and the accuracy of the estimate with an ensemble of control neurons increased. By contrast, the estimate with an ensemble of CA1-KO neurons, while improved to some extent, remains quite poor, making large errors (Fig. 4B,C). Cells that ought to fire together because they are tuned to similar locations, do not robustly do so in the knockout mice. Thus, in CA1-KO mice the ability of the animal to use a hippocampal ensemble code as a robust indication of spatial location is radically impaired. 'Hebbian' learning rules operating in downstream brain regions will fail to learn anything about place from CA1 in the knockout mice. We believe that this explains the navigational deficit observed in these animals.

## Concluding remarks and perspectives

In conclusion, we have demonstrated that a genetically engineered mouse strain can be generated in which a

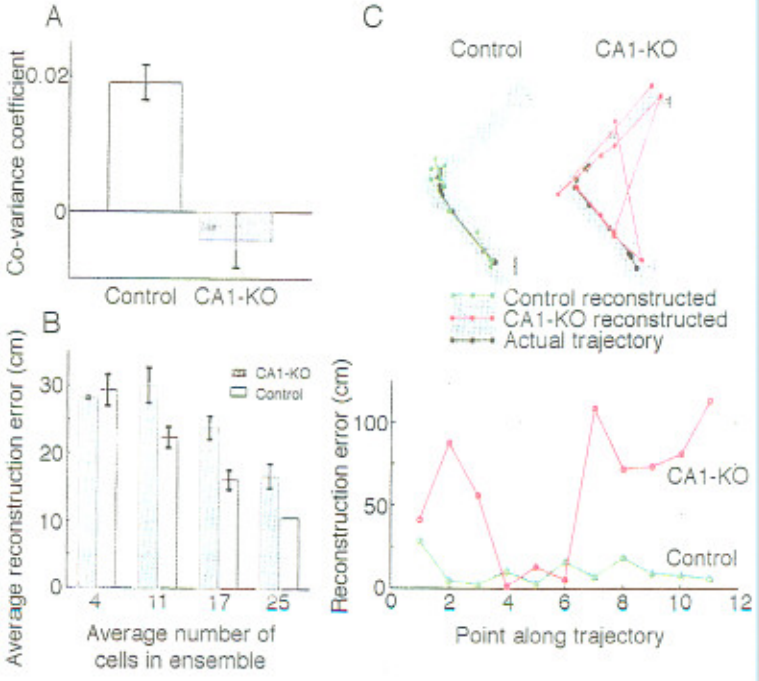


Fig. 4. *Ensemble coding properties of CA1 pyramidal cells in NMDA$_1$-receptor CA1 knockout (CA1-KO) and control mice.* (A) *The average co-variance coefficient of firing rates between overlapping pairs of control and pairs of knockout pyramidal cells. Pairs of cells in knockout animals fired randomly with respect to each other when their place fields overlapped.* (B) *Average error in path reconstruction. Trajectory reconstruction error is larger in CA1-KO mice. With few simultaneously recorded cells there was no significant difference between knockouts and controls. With large numbers of cells, the chance of overlapping fields increased, and the lack of co-variance in the knockouts appeared as an increased reconstruction error compared to controls. The position was reconstructed every 2 s by comparing a list of the average firing rate of each cell for a 2 s bin with a list of the average rates over the entire session, and finding the position that gave the closest match.* (C) *Examples of trajectory reconstruction. The upper panel illustrates trajectories reconstructed for control and CA1-KO animals for a 20 s stretch of behavior. The ensemble firing of place cells of knockouts does not coincide with the actual location of the animal. Points indicate locations at which position estimates and measurements were made. Lines connect successive points in time. Each arm of the L-track was 75 cm long. The lower panel shows the differences between the reconstructed and actual locations for the same data. The knockouts had a highly variable reconstruction error with occasional large values.*

gene of interest is deleted postdevelopmentally only in a limited area or in a particular cell type in the brain. This resolves the major drawbacks of the conventional gene-knockout technology and allows an identification of the role of a specific gene product utilized in a specific area or cell type of the adult brain during an animal's behavior and cognition. We applied this new knockout technology to the NMDA receptors in the hippocampal CA1 pyramidal cells in order to dissect the molecular and cellular mechanisms underlying the acquisition of spatial memory. By characterizing the CA1 region-restricted, $NMDA_1$-receptor knockout mice with a spatial memory task and with electrophysiology of hippocampal slices, we discovered a crucial link between NMDA receptor-dependent synaptic plasticity and spatial memory. By applying *in vivo* multiple-electrode recording techniques to the mutant mice, we found that the establishment of refined internal spatial codes in the CA1 region depends on the NMDA receptor-dependent synaptic plasticity. It is most likely that the impaired spatial memory observed in the mutant mice is attributable to the lack of refined, internal spatial codes.

In the coming years, a number of *Cre* transgenic lines with different regional or cell-type specificities will be produced. Furthermore, temporal specificity can be added to the regional or cell-type specificity by combining it with, for example, the recently reported gene-induction system based on the tetracycline repressor or operator system, or other inducible systems[20–22]. These genetically engineered mice can be analysed with a variety of techniques designed to identify abnormalities occurring at different levels of complexity – single synapse, single cell, cell ensemble and behavior. Because of the regional and temporal specificity of the genetic manipulation, this approach will allow identification of causal relationships between mechanisms at each of these levels.

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