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Inactivating the activated: identifying functions of specific neural networks

Rachel J Smith & Gary Aston-Jones

Manipulation of the neurons required for a specific behavior can be difficult, but is required for proof of causality. A clever technique now allows inactivation of only the subset of neurons that have been recently active.

In the carnival game 'Whack-a-mole', the aim is to knock down all of the moles that pop up from an array of holes in a console in front of you. Although you can't reach all of the moles, the active ones are vulnerable to attack. In this issue, Koya *et al.*[1] use a similar idea to develop a method to manipulate only the neurons that are critical for a particular behavior. Previous studies trying to prove the importance of a particular group of neurons have used the classical approaches of electrolytic/neurochemical lesions, as well as local microinjections of pharmacologic agents and electrical/chemical stimulation. Investigators can now also make use of targeted toxins for neurochemical-specific lesions, selective receptor agonists/antagonists, transgenic animals and promoter-specific viruses capable of inserting select genes. However, for the most part, these manipulations target all of the neurons with a given profile in a brain region, regardless of their activity or involvement in a particular behavior. Koya *et al.*[1] now present a technique that advances the field one step closer to the goal of activity-dependent manipulation. Using an approach termed the Daun02-inactivation method, the authors were able to selectively inactivate a subset of neurons that are activated during expression of cocaine-induced locomotor sensitization and to demonstrate that specific subsets of neurons in the nucleus accumbens are necessary for this context-dependent learned behavior.

Repeated administration of drugs can cause tolerance or sensitization to specific drug effects. Both of these phenomena can be influenced by the context in which the drug is administered[2], as a result of attachment of motivational importance to previously neutral environmental stimuli following repeated associations with the drug[3]. For example, context-specific cocaine-induced psychomotor sensitization is a progressive increase in cocaine-induced locomotion in rats following repeated drug exposure in a particular environment[4]; rats previously exposed to cocaine in one environment do not show locomotor sensitization when cocaine is administered in an alternative environment.

Dopaminergic projections to nucleus accumbens have been implicated in cocaine-induced locomotor sensitization[5]; however, only a small subset (2–3%) of accumbal neurons shows activation related to context-specific sensitization[6]. Previous research using histochemistry and behavioral electrophysiology/electrochemistry indicates that this small population of neurons may represent a neuronal ensemble, a group of cells with a distinct set of functional properties that are activated by specific inputs[6,7]. This sparsely distributed subset of accumbal neurons is proposed to encode the learned association between drugs of abuse and the environment in which they are administered, but this hypothesis has not been directly testable with the available methods.

In the study by Koya *et al.*[1], cocaine was administered to rats for 7 d in a novel environment. After 7 d of withdrawal, cocaine or saline was injected in the same paired environment and a sensitized locomotor response was observed for cocaine only when given in the paired environment, as expected. Examination of brain tissue revealed that a subset of accumbal neurons expressed Fos (a protein encoded by the *c-fos* gene, and a marker of neural stimulation) following this context-specific cocaine-induced locomotor sensitization. The authors hypothesized that these stimulated neurons might instantiate the context-related conditioning that resulted in locomotor sensitization to cocaine.

To test this hypothesis, the authors set out to selectively inactivate only those neurons that expressed Fos. To do this, they combined two previous developments: *c-fos–lacZ* transgenic rats[8] and the anthracycline prodrug Daun02 (ref. 9). Daun02 is converted by β-galactosidase to daunorubicin, a cytotoxic antitumor agent that kills cells and has also been reported to electrically silence cells. In *c-fos–lacZ* transgenic rats, neuronal activity stimulates the *c-fos* promoter in the transgene, driving expression of the bacterial *lacZ* gene that encodes the protein β-galactosidase. Local microinjection of Daun02 then leads to production of daunorubicin in cells that contain β-galactosidase, leading to presumed long-lasting inactivation or death of Fos-expressing neurons.

In these Daun02 studies, *c-fos–lacZ* transgenic rats were conditioned with cocaine in a novel environment for 7 d (**Fig. 1**). These rats received an injection of cocaine or saline in the paired environment 7 d later (to induce both a sensitized response and Fos and β-galactosidase expression) and received intra-accumbal infusions of Daun02 90 min after that (induction day). Cocaine was again injected in the same paired environment 3 d later (test day). Rats that were given intra-accumbal Daun02 on the cocaine induction day had attenuated cocaine-induced locomotion on the subsequent test day. Locomotor levels

The authors are in the Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina, USA.
e-mail: smithrac@musc.edu or astong@musc.edu

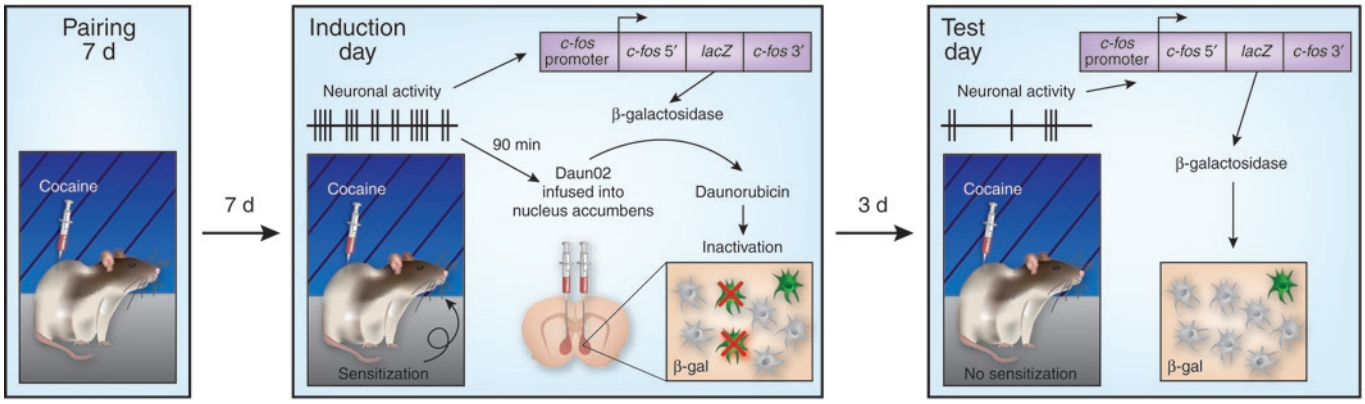

Figure 1 Koya *et al.*[1] developed the Daun02-inactivation method to demonstrate that a specific subset of activated neurons in nucleus accumbens is involved in context-specific cocaine-induced locomotor sensitization. *c-fos–lacZ* transgenic rats were repeatedly exposed to cocaine in a novel environment for 7 d (pairing days, left). Following 7 d of withdrawal, a cocaine injection in the paired environment resulted in a sensitized cocaine-induced locomotor response and neuronal activation in a specific subset (2–3%) of accumbal neurons (induction day, center). In activated neurons, the *c-fos* promoter transgene drove expression of the *lacZ* gene, resulting in β-galactosidase production. Local microinjection of the prodrug Daun02 into nucleus accumbens 90 min after the behavioral response resulted in production of daunorubicin (converted by β-galactosidase), which inactivated or lesioned the activated neurons. When cocaine was again administered in the paired environment 3 d later (test day, right), there was a complete loss of cocaine-induced locomotor sensitization and a 50% reduction in β-galactosidase–expressing neurons, indicating that Daun02 caused inactivation of the specific subset of accumbal neurons that is necessary for the learned association between cocaine and the environment.

were comparable with those observed on the first day of cocaine exposure in the paired environment, indicating a complete loss of sensitization. In these same Daun02-treated rats, cocaine-induced β-galactosidase expression was reduced by 50% on later tissue analysis, consistent with a loss of activated neurons. These behavioral and neural responses were not observed in transgenic rats given Daun02 dorsal to the accumbens or given intra-accumbal vehicle injections, or in wild-type rats given intra-accumbal Daun02. Notably, Daun02 injections following saline administration on the induction day had no effect on subsequent cocaine-induced locomotion. This is consistent with their observations that exposure to the environment alone is not sufficient to induce Fos expression in the accumbal ensemble and that Daun02 does not cause nonspecific inactivation. These results lead the authors to speculate that Daun02 inactivated a subset of accumbal neurons that specifically encoded the learned association between cocaine and the paired environment, resulting in a loss of context-specific cocaine-induced locomotor sensitization.

If further validated with additional behavioral procedures, this new technique provides the opportunity to investigate the causal role of activated neurons (even if sparsely distributed) in specific behaviors. This approach can be used to determine whether neuronal ensembles such as these are responsible for encoding other types of learned behavior and (if the Daun02 effect on activated neurons is permanent) whether new populations of neurons can be recruited for a given behavior if the first subset is inactivated or lesioned. The elegance of the technique is that the effect is specific for neurons that are activated during a behavior, and, therefore, pinpoints the subset of neurons that is thought to be involved in encoding the learned response. It can be potentially useful in a variety of fields that are interested in relating the activity of small populations of neurons to a larger function such as behavior and can be critical for moving past correlative studies that are based on Fos and electrophysiological activation and on to causal relationships between specific neurons and behaviors.

However, as with most new techniques, there are still several questions to be answered relating to the mechanism of Daun02 inactivation. These studies indicate that the effects of Daun02 last at least 3 d, but does daunorubicin cause reversible inactivation or cell death? In addition, how effective is Daun02 for inactivating Fos-expressing cells? β-galactoside expression was reduced by only 50%, even though cocaine-induced locomotor sensitization appeared to be completely blocked. Therefore, the potency of Daun02 to inactivate or lesion Fos-expressing neurons in this model is unknown, perhaps limiting a tight association between activated neurons and behavior with this method.

Future studies will undoubtedly examine the long-lasting behavioral effects of inactivating a specific subset of accumbal neurons. It is unknown whether rats previously subjected to Daun02 inactivation for blockade of cocaine-induced locomotor sensitization would be able to exhibit future sensitization. It is also important to determine whether this technique can be used in other brain regions. Does Daun02 inactivation require specific neuronal qualities, such as Fos expression exclusively in a subset of neurons related to the function being studied (for example, behavioral association), expression of a substantial level of Fos in response to neural activity or low levels of basal Fos expression? Answers to questions such as these will help validate the usefulness of this potentially powerful technique.

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