Archival Report

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Associative Learning Drives the Formation of Silent Synapses in Neuronal Ensembles of the Nucleus Accumbens

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ABSTRACT

BACKGROUND: Learned associations between environmental stimuli and rewards play a critical role in addiction. Associative learning requires alterations in sparsely distributed populations of strongly activated neurons, or neuronal ensembles. Until recently, assessment of functional alterations underlying learned behavior was restricted to global neuroadaptations in a particular brain area or cell type, rendering it impossible to identify neuronal ensembles critically involved in learned behavior.

METHODS: We used Fos-GFP transgenic mice that contained a transgene with a Fos promoter driving expression of green fluorescent protein (GFP) to detect neurons that were strongly activated during associative learning, in this case, context-independent and context-specific cocaine-induced locomotor sensitization. Whole-cell electrophysiological recordings were used to assess synaptic alterations in specifically activated GFP-positive (GFP+) neurons compared with surrounding nonactivated GFP-negative (GFP−) neurons 90 min after the sensitized locomotor response.

RESULTS: After context-independent cocaine sensitization, cocaine-induced locomotion was equally sensitized by repeated cocaine injections in two different sensitization contexts. Correspondingly, silent synapses in these mice were induced in GFP+ neurons, but not GFP− neurons, after sensitization in both of these contexts. After context-specific cocaine sensitization, cocaine-induced locomotion was sensitized exclusively in mice trained and tested in the same context (paired group), but not in mice that were trained in one context and then tested in a different context (unpaired group). Silent synapses increased in GFP+ neurons, but not in GFP− neurons from mice in the paired group, but not from mice in the unpaired group.

CONCLUSIONS: Our results indicate that silent synapses are formed only in neuronal ensembles of the nucleus accumbens shell that are related to associative learning.

Keywords: Addiction, Electrophysiology, Glutamate, Memory, Psychostimulant, Transgenic

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Learned associations between drugs of abuse and environment stimuli play a critical role in drug addiction. One of the key aspects of these drug-related memories is that one specific stimulus can induce a conditioned drug behavior such as relapse, whereas other unrelated stimuli do not. The neurobiological mechanism that underlies this type of learning must encode these drug-related memories with a degree of resolution sufficient to discriminate between memories activated by different sets of stimuli. Recent studies support the hypothesis that specific patterns of sparsely distributed Fos-expressing neurons act together as neuronal ensembles that mediate addiction-related learned behaviors (1–6), including context-specific expression of reinstatement of drug seeking (1,2), incubation of drug craving (3), and context-specific locomotor sensitization (4). These Fos-expressing neuronal ensembles are formed by only a few neurons (~2%–12%) that are selected by the relevant cues. The enormous number of possible permutations of these neuronal ensembles provides sufficiently high resolution to discriminate between different associative memories. Selective alterations within these neurons are likely to play a key role in learning and maintenance of these drug-related memories.

Synaptic plasticity is the key candidate neural mechanism for encoding learning and memory processes. Many years of research have shown that exposure to cocaine and other drugs of abuse can induce synaptic alterations in reward-related brain areas such as the nucleus accumbens and ventral tegmental area (7–11). These neuroadaptations include alterations in the balance of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor–mediated and *N*-methyl-D-aspartate receptor–mediated transmission (7,9), changes in AMPA receptor subunit composition (12,13), alterations in synaptic plasticity (11,14), and the development of silent synapses (15,16). Synaptic alterations in

addiction-relevant brain areas have also been shown to play an important role in the development and persistence of behavioral models in addiction research (17–19). However, these synaptic alterations are observed in whole-brain areas or in particular cell types regardless of their neural activity during the learned behaviors. Although these global alterations can play important general roles in learning and maintenance of memories, other mechanisms are required to encode the high-resolution information in cue-specific memories and discriminate them from other memories stored in the same brain area. Based on previous studies from our laboratory and others, we hypothesize that unique synaptic alterations induced within drug cue–activated Fos-expressing neuronal ensembles play a critical role in the expression of many learned associations in conditioned drug behaviors (5,6).

We used Fos-GFP transgenic mice that contained a transgene with a Fos promoter driving expression of green fluorescent protein (GFP) to detect neurons after context-specific sensitization of cocaine-induced locomotion sensitization (20). The small proportion of GFP-expressing neurons that was strongly activated during sensitized cocaine-induced locomotion exhibited large percentage increases of silent synapses and related synaptic alterations that were not present in most surrounding GFP− neurons that were less activated during behavior. We hypothesized that the emergence of silent synapses might represent a cellular mechanism contributing to the development of learned associations, but we were unable to determine this because mice were trained and tested in the same context. Under these circumstances, the development of silent synapses could result from a compensatory response of neurons that were particularly strongly activated by repeated exposure to cocaine with no role in associative learning. To address whether silent synapses are induced in a context-specific manner, we examined synaptic alterations in GFP+ and GFP− neuronal populations after two different forms of cocaine-induced locomotor sensitization: 1) context-independent form of sensitization in which animals displayed a sensitized locomotor response regardless of their previous training context and 2) context-specific form of sensitization in which mice were able to distinguish between two different contexts and displayed a sensitized locomotor response only when tested in the context in which they were trained.

METHODS AND MATERIALS

Animals

We used 163 male and female Fos-GFP transgenic mice in our experiments. These mice were initially obtained in 2008 from Alison Barth at Carnegie Mellon University. We bred male heterozygous Fos-GFP mice with female wild-type C57BL/6 mice (Charles Rivers Laboratories International, Inc., Wilmington, Massachusetts) for >15 generations at the Intramural Research Program facilities of the National Institute on Drug Abuse. An additional 24 male wild-type C57BL/6 mice were obtained from Charles Rivers Laboratories. Mice were maintained in a temperature-controlled and humidity-controlled facility on a 12-hour light-dark cycle. Mice were separated and individually housed with ad libitum food and water for 3–5 days before training. All experiments were conducted during the light phase. Animal protocols were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program and were carried out according to U.S. National Institutes of Health Guidelines.

Behavioral Procedures

Context-Independent Sensitization. Male and female Fos-GFP mice (n = 47) were habituated three times once daily for 60 minutes each day in locomotor activity chambers (43 × 43 cm, Med Associates, Inc., St. Albans, Vermont) and then divided randomly into two groups with approximately equal numbers of male and female mice. Mice in the paired group were injected once daily for 5 days with cocaine (15 mg/kg intraperitoneal [i.p.]) or saline (10 μL/g) in the locomotor activity chamber (Context A). Mice in the unpaired group were injected once daily for 5 days with cocaine (15 mg/kg i.p.) or saline (10 μL/g) in a different context (Context B-Independent), which was circular and contained a grid floor. On test day, after 6–11 days in their home cages, all mice were given a single injection of cocaine (20 mg/kg i.p.) in Context A and perfused 90 minutes later.

Context-Specific Sensitization. Male and female Fos-GFP mice (n = 116) were divided randomly into two groups with approximately equal numbers of male and female mice. Mice in the paired group were injected once daily for 5 days with cocaine (15 mg/kg i.p.) or saline (10 μL/g) in the locomotor activity chamber (Context A). Mice in the unpaired group were injected once daily for 5 days with cocaine (15 mg/kg i.p.) or saline (10 μL/g) in a different context (Context B-Specific), which was a round bowl with bedding and a toy; lights were dimmed and music (No Doubt, "Tragic Kingdom") was played continuously in this context. On test day, after 6–11 days in their home cages, all mice were given a single injection of cocaine (20 mg/kg i.p.) in Context A and perfused 90 minutes later. In a separate experiment, 24 male C57BL/6 mice received identical training, but on test day, half the mice received cocaine injections (20 mg/kg), and half received saline injections (10 μL/g).

Immunohistochemistry

Fos-GFP mice were transcardially perfused 90 minutes after the final injection of cocaine on test day with 4% paraformaldehyde. Fos immunohistochemistry and Fos and neuronal nuclear antigen (NeuN) double-labeling were performed as previously described (4). For detailed information, see the Supplement.

Ex Vivo Brain Slice Electrophysiology

On test day, Fos-GFP mice were deeply anesthetized with isoflurane (60–90 s) 90 minutes after cocaine injections and transcardially perfused with ice-cold cutting solution. Coronal brain slices containing nucleus accumbens shell were prepared, and whole-cell voltage-clamp recordings were performed in GFP+ and GFP− medium spiny neurons of the nucleus accumbens shell as previously described (20). Spontaneous excitatory postsynaptic current (EPSC) data were collected using WinEDR software (courtesy of J. Dempster, University of Strathclyde, Glasgow, United Kingdom) and