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Resisting the urge to act: DREADDs modifying habits

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Abstract

Recently, Meyer and Bucci used chemogenetic technology - artificial excitatory and inhibitory receptors - to modulate neuronal activity in two connected brain regions in opposite directions simultaneously. This innovative manipulation revealed that the two regions studied, orbitofrontal cortex and nucleus accumbens, are not sequentially dependent during contextual decision-making.

Habit learning is a form of conditioned response to a stimulus. Following habit learning, it is often harder to learn to suppress the conditioned response, when appropriate, than it was to learn the association in the first place. For example, you may have grown accustomed to inserting several coins into a vending machine, pressing the backlit button sporting the logo representing your beverage of choice, and retrieving said beverage from the dispenser.

However, you have likely learned that when the small red light adjacent to the button is also lit, you should withhold your intention to press the button, because the beverage you desire is currently unavailable, and any attempt to request it will likely result in the machine eating your money.

Meyer and Bucci recently used a chemogenetic approach, termed designer receptors exclusively activated by designer drugs (DREADDS) (see Text Box 1), to reveal part of the neural circuitry involved in learning to withhold a behavioral response in the face of a secondary stimulus that modifies the meaning of a previously learned association [1]. They used an excitatory DREADD to increase activity of neurons in the rat nucleus accumbens (NAC) upon systemic delivery of the DREADD ligand, clozapine-N-oxide (CNO), and an inhibitory DREADD to silence cells, again, upon delivery of CNO, in the orbitofrontal cortex (OFC). A chemogenetic approach was particularly well suited to this study, because a similar design attempting to use a pharmacological or optogenetic approach would have required repeated instrumentation of each of the bilaterally targeted regions, which would not only have compromised the integrity of the tissue under study, but also that of the regions overlying these deep structures. The chemogenetic approach also has the obvious advantages over permanent lesions that it considerably reduces the likelihood of compensatory plasticity generating a false-negative result, or damage to fibers of passage producing a false positive.

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Meyer and Bucci's clever experimental design used inhibitory and excitatory DREADDs to silence OFC and excite NAC simultaneously while rats performed a contextual decision making task. In their task, rats learned to associate a tone with the availability of food pellets (analogous to the way a human might associate a button on a beverage dispenser with the pleasure of drinking the beverage represented). Learning was measured by the amount of time the rats spent approaching a small receptacle in which food pellets were delivered after the presentation of the tone. A light was presented on 25% of trials *before* the presentation of the tone – this indicated that the trial would *not* be rewarded. The authors' intention was for the presentation of the light to be equivalent to the illumination of the red diode on the beverage button indicating that no drink is available for pressing the button on this occasion. However, a better analogy would be that the whole machine was switched off on this subset of trials. In order to demonstrate a specific effect on contextual decision making, i.e., to conclude that the light stimulus affected the rats' interpretation of the auditory tone, the authors would have needed to include a third set of trials in which reward *was* delivered when the presentation of the light was followed by a third stimulus (e.g. a different tone). Without this control condition the light can be considered similar to a 'blocking' signal [2], i.e. it indicates not only that the tone should be ignored on this trial, but that all stimuli subsequent to the presentation of the light should be ignored. It will be interesting to see if future studies including this control condition show similar effects to those reported in [1].

Given a recent report that, in some strains of rat, CNO can modulate behavior in the absence of DREADDs [3], it is important to note that rats that had received control virus injections were unaffected by systemic CNO injections; that is, they spent more time approaching the food receptacle on trials in which the tone alone was presented (food available) than on trials in which the light preceded the tone (no food available). Rats that had received an injection of virus into NAC that resulted in localized expression of excitatory hM₃Dq receptors spent more time approaching the receptacle on unrewarded trials after CNO administration than did their control counterparts. This effect was specific to excitation of the NAC, as the authors report that silencing of this region did not alter approach behavior. Rats that had OFC transduced with an inhibitory DREADD, hM4Di, also spent more time approaching the food receptacle on unrewarded trials following CNO administration than their control counterparts. Thus either excitation of NAC or silencing of OFC was sufficient to impair contextual decision-making (i.e. the rats were doing the equivalent of pressing the button to request a beverage, even though the machine was switched off). The final component of the experiment was particularly interesting: Meyer and Bucci transduced NAC with the excitatory DREADD and OFC with the inhibitory DREADD in the *same* animals. Systemic administration of CNO simultaneously activated NAC and silenced OFC in these subjects. The effect on behavior was approximately the sum of modulating these areas independently (see [1] Fig. 3).

It has been proposed that low PFC activity and concurrent high NAC activity, as occurs during human adolescence, could contribute to heightened impulsivity and risk-taking [4, 5]. Meyer and Bucci claim to have causally tested this hypothesis by disrupting the balance between PFC and NAC by simultaneously modulating the activity of both regions using DREADDs. However, to demonstrate clearly that a balance between OFC and NAC is required for appropriate contextual decision-making, it would have been necessary to

observe a non-linear effect of simultaneously modulating these two regions; e.g. a synergistic effect on disruption of behavior, or even a sub-linear effect – anything that can be interpreted as a statistically significant interaction. Thus, the most parsimonious interpretation of the result obtained is that OFC and NAC do not interact sequentially, rather they likely exert their effects on context-dependent decision making via a final common pathway.

The function of the direct projection from PFC to NAC could be explored using a chemogenetic technique with greater precision; the two-component DREADD system - the 'DiO' (Double-floxed inverse orientation) or 'FLEX' (flip-excision) system [6] - allows for the injection of one component in one region, and the other in an interconnected region. The outcome is that only the neurons projecting from the first region to the second will express the DREADD receptor [7]. Initially one could inject only the anterograde expressing component of the two-component system, and look for regions of dense projections. Some of the most promising candidate areas could then be selected for the functional connectivity study. This process could be further refined to exploit another advantage of the DREADD technology; by using cell-specific promoters, one could target only the projections of cells that release a specific neurotransmitter. For example, the tyrosine hydroxylase (TH) promoter fragment limits expression to dopamine/noradrenaline producing cells [8] (see [9] for review). Nonetheless, the approach used by Meyer and Bucci serves as one blueprint for studying the distributed networks thought to support contextual decision-making or other clearly defined behaviors.

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Text box 1**Chemogenetic tools**

The term ‘chemogenetics’ applies to any technology that introduces genetic material encoding an artificial receptor that, once expressed, can be activated by administration of an exogenous ligand, either systemic or local. Currently, the most commonly used such system is the Designer Receptor Exclusively Activated by a Designer Drug (DREADD) set of receptors developed by Bryan Roth and colleagues [10]. DREADDs can be either excitatory or inhibitory. The hM₃Dq DREADD is a G-protein coupled receptor (GPCR) that interacts with a G_q protein; activation of this receptor results in heightened excitability of the cell membrane, and increased firing rates in neurons. The inhibitory DREADD, hM₄Di, is Gi-coupled; activation leads to neuronal hyper-polarization and, independently, a reduction in the probability of presynaptic neurotransmitter release [11].