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## Using Optogenetics to Study Habits

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### Abstract

It is now well documented that optogenetics brings to neuroscience a long sought-after foothold to study the causal role of millisecond-scale activity of genetically or anatomically defined populations of neurons. Progress is rapid, and, as evidenced by the work collected in this Special Issue, the possibilities of what can now be done are almost dizzying. Even for those concerned with complex phenomena, such as behavioral habits and flexibility, signs are that we could be on the threshold of a leap in scientific understanding. In this article, we note this special time in neuroscience by the example of our use of optogenetics to study habitual behavior. We present a basic sketch of the neural circuitry of habitual behavior built mainly on findings from experiments in which lesion and drug microinjection techniques were employed in combination with sophisticated behavioral analysis. We then outline the types of questions that now can be approached through the use of optogenetic approaches, and, as an example, we summarize the results of a recent study of ours in which we took this approach to probe the neural basis of habit formation. With optogenetic methods, we were able to demonstrate that a small site in the medial prefrontal cortex can control habits on-line during their execution, and we were able to control new habits when they competed with prior ones. The nearly immediate effect of disabling this site optogenetically suggests the existence of a mechanism for moment-to-moment monitoring of behaviors that long have been thought to be almost automatic and reflexive. This example highlights the kind of new knowledge that can be gained by the carefully timed use of optogenetic tools.

### Keywords

Basal ganglia; prefrontal cortex; reward; learning; plasticity; executive; time

## 1. Introduction

William James wrote that, “Ninety-nine hundredths or, possibly, nine hundred and ninety-nine thousandths of our activity is purely automatic and habitual, from our rising in the morning to our lying down each night” (James, 1899). Some might think that this view overstates the presence of habits, given modern definitions of habitual behavior. Yet, at the heart of the statement lies truth: habits, rituals and routines are pervasive, powerful and familiar parts of our lives, and have been points of great scientific interest for over a century. Now, work on the neural basis of habit formation has given us a blueprint for the brain circuits that are engaged as habits arise, and the beginnings of an idea of how they are

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represented in activity patterns. This work has proven critical to the study we review here, in which we took advantage of optogenetic approaches to evaluate the on-line mechanisms for habits (Smith et al., 2012). The fine temporal resolution, gene-based targeting strategies, and repeatability of optogenetic manipulations gives the opportunity to intervene causally in the brain's activity at a millisecond level and with cell-type specificity (Bernstein and Boyden, 2011; Fenno et al., 2011). It is now possible to address long-standing questions about when during learning and performance habits are selected and controlled, and which neural circuits are necessary and sufficient for habits to be acquired and expressed. In addition, classic ideas about habits can be probed by repeating manipulations over time, including the idea that habits, once deeply engrained, can almost never be totally forgotten (Pavlov, 1927). Our first work with optogenetic methods touches on these issues, but especially, along with related work on the neural basis of addiction, underscores the potential of optogenetic approaches to this field.

## 2. Habits: brain substrates and conceptual frameworks

A major substrate for habitual behavior is known to depend on basal ganglia-related circuits with key nodes in the sensorimotor region of the striatum (the dorsolateral striatum, typically abbreviated as DLS). This region is a central component of circuits critical for building representations of sequences of often repeated behavior, whether learned or innate, into action patterns (Aldridge et al., 2004; Brainard and Doupe, 2002; Carelli et al., 1997; Fee and Goldberg, 2011; Graybiel, 2008; Hikosaka and Isoda, 2010; Poldrack et al., 2005; Yin et al., 2009). Such action-sequencing is adversely affected in neurologic disorders such as Parkinson's disease, for which initiating, conducting, and ending even simple sequences of movement become challenging. In other disorders, including those related to obsessive-compulsive disorder, sequences of behavior are excessively repeated. Dysfunctions in the basal ganglia appear to underlie many aspects of these conditions.

An important conceptual advance in the field was to provide conditions under which such action-sequences could be understood as habits. Even though habits are expressed as fast and sometimes skilled action sequences, such action-sequences are not necessarily habitual. Learning theory suggests that habits emerge from a change in covert strategy alongside the observable, overt refining of behaviors that occurs as they are repeated. For example, navigational behaviors dependent on reinforced action learning can be driven by habitual response plans (e.g., run straight then turn left) or, instead, can be triggered by external cues (e.g., approach that wall, approach that food dish) (Packard, 2009; Tolman et al., 1947). A simple test has been designed to pit these two alternatives against one another by rotating the task apparatus 90-degrees after a learning phase, without moving the cues, and then determining whether an animal follows the cues or emits the learned response (McDonald and White, 1993; Packard and McGaugh, 1996; Tolman et al., 1947). A response-based (egocentric) strategy is thought to represent an ingrained habitual form of behavior, as it is fully dissociated from Pavlovian cue approach or related stimulus-directed behaviors, and can emerge as a dominant strategy with repeated running or can be instantiated early if task conditions require it (Packard, 2009).

A similar distinction in the underlying behavioral strategy comes from associative learning frameworks of habitual and goal-directed action (Balleine and Dickinson, 1998; Dickinson, 1985; Hull, 1943; Thorndike, 1898). By these accounts, habits are driven by learned stimulus-response associations, and they can be distinguished from behavior based on learned action-outcome associations. A particular behavior – say, pressing a lever – can be driven by either of these two very different underlying processes, and yet appear identical or nearly so. Which of these learning rules is being used to perform behavior can be determined, for example, through manipulations of the learned outcome value. Behavior

based on action-outcome associations is sensitive to this manipulation (i.e., is goal-directed), whereas behavior rooted in stimulus-response links is reflexive and insensitive (i.e., is habitual) (Adams, 1982; Balleine and Dickinson, 1998; Dickinson, 1985; Yin and Knowlton, 2006). This differentiation made by psychologists has influenced contemporary computational models of learning and behavior, notably the analogous proposal that the brain contains separate learning systems specialized for purposeful behavior based on predictions derived from a model of the task environment (i.e., model-based) or behavior based on history and the state-dependent values of behavior that have been stored (i.e., model-free, analogous to habits) (Bornstein and Daw, 2011; Daw et al., 2005a; Daw et al., 2005b).

In neurobiology, studies based on these frameworks implicate the DLS and associated basal ganglia-related circuits as important not only for the performance of sequential behaviors, but also for behaviors that are outcome-insensitive and response-based (Packard, 2009; Yin et al., 2004). Additional regions promoting habits have been identified, and, with the DLS, they are thought to form parts of functional networks (Faure et al., 2005; Lingawi and Balleine, 2012; Nelson and Killcross, 2006; Wang et al., 2011; Yin and Knowlton, 2006). These networks contrast with others including the dorsomedial, associative, striatum (DMS) and limbic circuitry, which are thought to promote behavioral flexibility, outcome-sensitivity, and the use of external cues to guide behavior (Balleine and O'Doherty, 2010; Packard, 2009; Ragozzino, 2007; Yin and Knowlton, 2006).

### 3. Mechanisms for the shift from flexible behavior to habits

Habit formation is a dynamic process. Many habits emerge out of initial exploration of environments, learning of responses, and sculpting of purposeful action plans. With repetition, behaviors then grow less flexible and more ingrained, becoming almost reflexive. Habit formation of this sort is thought to involve plasticity not only in habit-promoting sites, but also in flexibility-promoting sites. In this way, habits might entail a tip in the balance between competing neural systems (Balleine et al., 2009; Daw et al., 2005a; Packard, 2009; Thorn et al., 2010; Yin and Knowlton, 2006). Human brain imaging studies, with the work of the Passingham group as an early example (Jueptner et al., 1997a; Jueptner et al., 1997b), as well as many other studies (Balleine and O'Doherty, 2010; Hikosaka et al., 2002; Poldrack et al., 2005; Graybiel, 2008), have shown changes in neural activity that coincide with this dynamic process, generally from anterior prefrontal to posterior frontal cortical regions and anterior to posterior striatum; and in rodents, work has implicated a comparable progressive shift in engagement of striatal regions from medial and anterior striatum (e.g., DMS) to more lateral and posterior striatum (e.g., DLS) (Belin et al., 2009; Graybiel, 2008; Willuhn et al., 2012; Yin et al., 2009). These transitions from flexible to habitual behavior have been our particular focus in a series of studies in which we have tracked with multiple electrodes the activity of ensembles of neurons in the striatum and neocortex as animals learn habits. In experiments in which rodents learn maze tasks, this work has identified the gradual emergence of a special action-bracketing pattern of ensemble activity in the DLS as habits are initially learned and then stamped in by extended training, as well as a shift in the balance of activity between the DMS and the DLS as these changes in behavior occur (Barnes et al., 2005; Barnes et al., 2009; Howe et al., 2011; Jog et al., 1999; Kubota et al., 2009; Smith and Graybiel, in submission; Thorn et al., 2010). For example, with simultaneous ensemble recordings of projection neurons in the DMS and DLS during habit learning, DMS activity strengthened around the decision points during the initial learning of the maze-tasks, but then, when these runs were practiced through extended training, this DMS activity waned (Thorn et al., 2010). By contrast, in the DLS, the ensemble activity was initially high during the maze runs, but as the task was learned, and the runs became fast and regular, activity quieted during most of the run-time but grew strong near the start and end.

This task-bracketing pattern was very strong even after the DMS activity decreased. The temporal alignment of the activity to near the beginning and end of the behavior can vary with task conditions (for example the activity can mark early and late action events, such as initiation and the final turning, and can be more or less temporally phasic), but across an increasing number of studies in rats and mice, the basic beginning-end pattern has appeared with learning.

We have suggested as a working hypothesis that this activity represents the “chunking” together of the run sequences into single, rapidly executable units (Graybiel, 1998; Graybiel, 2008). Along with this task-bracketing, some neurons become highly tuned to individual parts of the maze runs. These could be “expert neurons” tiling the task-time (Barnes et al 2005). This pattern of activity has also been observed in the prefrontal cortex and striatum of macaques performing well practiced sequences of saccades (Fujii and Graybiel, 2003), in several sites as rodents repeatedly tap a lever (Jin and Costa, 2010), and in the HVC of Bengalese finches during song repetitions (Fujimoto et al., 2011). This agreement suggests that task-bracketing is a conserved neural instantiation of crystalized action plans across multiple species and brain regions.

How is this *shift* in strategy, from goal-directed to habitual, controlled in the brain? The existence of multiple action systems that appear to compete with one another for expression has raised the possibility that some mechanism could be in place for selecting or arbitrating among them (Daw et al., 2005a; Killcross and Coutureau, 2003; Wunderlich et al., 2012; Yin and Knowlton, 2006). One candidate for such functions is the medial prefrontal cortex, particularly the medial cortical region that in rodents is called the infralimbic (IL) region. Lesions or pharmacological manipulations of this neocortical site revert habitual behaviors to an outcome-sensitive state, indicating that the IL cortex promotes habit formation similarly to the DLS (Coutureau and Killcross, 2003; Hitchcott et al., 2007; Killcross and Coutureau, 2003) (Fig. 1A). This IL site provides a functional contrast to its dorsal neighbors, the prelimbic and cingulate cortical regions, which appear to promote goal-directedness (Balleine and Dickinson, 1998; Killcross and Coutureau, 2003). Anatomically, the IL cortex itself projects chiefly to flexibility-promoting regions, including the prelimbic cortex and the DMS, but IL outputs also can indirectly reach the sensorimotor network (perhaps through the central nucleus of amygdala, shown to interact with the DLS for habits (Lingawi and Balleine, 2012)). These patterns of connectivity suggest that the IL cortex could influence the balance between interacting networks promoting flexible and habitual modes of behavior (Daw et al., 2005a; Hitchcott et al., 2007; Killcross and Coutureau, 2003). Consistent with such a function, we are finding in on-going work that changes in the pattern of activity of IL neurons closely correspond to entry into, and exit from, states of habitual performance (Smith and Graybiel, in submission).

#### 4. Halorhodopsin-mediated inhibition of IL cortex blocks habits on-line

We have applied optogenetics to ask directly whether the IL cortex might work as an executive on-line control system for habits (Smith et al., 2012). Our strategy for this initial work was to perturb the activity of IL pyramidal cells using halorhodopsin (eNpHR3.0; a light-sensitive membrane chloride pump that leads to cellular hyperpolarization when activated by yellow light) (Gradinaru et al., 2010). Light could then be delivered only during habit performance in order to establish a causal link between the activity of IL neurons and habit execution, in real time. This approach permits us to manipulate IL activity abruptly and briefly, specifically during the performance of the habit (not during pre- or post-run times). Moreover, we could re-apply the optogenetic intervention over weeks of experimental study, and thus over multiple time-points after the initial disruption of cortical activity. With this approach, we were able to study the functional impact of IL activity in

exerting real-time control over habits, as well as its role in selecting between multiple habits built up over long periods of time.

We first introduced into the IL cortex a virus containing a construct coding for halorhodopsin (AAV5-CaMKII $\alpha$ -eNpHR3.0-eYFP) (see (Smith et al., 2012) for additional details). We confirmed that delivery of light onto IL neurons could modulate their activity in a time-locked fashion (Goshen et al., 2011; Gradinaru et al., 2010; Tye et al., 2011). To do this, we developed a modified recording head-stage permitting light delivery onto tetrodes recording neural spiking activity in freely moving rats (Fig. 1B). When yellow light was delivered, we observed a more than 50% reduction in firing on average, with some neurons being inhibited nearly in full while others showing less complete inhibition. The result was a more moderate suppression of spiking compared to what might be expected from lesion or chemical inactivations. Importantly, these effects were stable for the ca. 3 sec illumination period and ca. 40 trials rats would receive in the task (Fig. 1C). Residual spiking increases after illumination offset, detected in some preparations but not others (Ferenczi and Deisseroth, 2012; Raimondo et al., 2012; Tonnesen et al., 2009), were not observed. However, the concurrent light delivery and tetrode recordings were important in demonstrating that the manipulation produced mixed results on physiological activity. Consistent with prior work (Anikeeva et al., 2011; Han et al., 2009), we found neurons with an increase in spike activity during light delivery, often recorded from the same tetrode as others that were inhibited by the light. At the population level, there was 1 cell showing excitation for every 1.8 cells showing inhibition. This result accords with known properties of cortical microcircuitry and the complex interaction of locally connected neurons driven by optogenetic perturbation, and accords with a general principle that when the activity of opsin-expressing neurons is altered, there will be reverberating effects on neurons that do not express the opsin or that fall outside of the illumination zone.

To assess the behavioral impact of this optogenetic perturbation of IL activity, we designed a T-maze task that allowed us to incorporate tests of outcome-sensitivity as measures of the degree to which the maze runs were habitual (Fig. 1D). In the task, rats waited at a start platform until a gate lowered. They then ran down the long-arm of the maze and were given one of two tone cues instructing them to turn down a right or left end-arm to receive a reward. Each end-arm had distinct reward (e.g., high frequency tone cue  $\rightarrow$  turn left  $\rightarrow$  receive chocolate milk if correct; low frequency tone  $\rightarrow$  turn right  $\rightarrow$  receive sugar water if correct). Rats were then extensively over-trained beyond the point of initial acquisition (Fig. 1E). We then administered home-cage reward devaluation of only one of the two rewards by applying a conditioned taste aversion protocol through pairings of reward intake with an injection of lithium chloride. This procedure resulted in a drastic reduction of reward drinking in the home-cage. Afterward, the rats were returned to the maze, and for one day we gave them a probe session in which the instruction cues played as normal, but correct performance did not result in reward (i.e., extinction conditions) (Fig. 1E). The lack of rewards allowed us to test whether they would still run to the side of the maze cued as having the now-aversive (devalued) goal, thus allowing us to estimate the extent to which the animals represented outcome value during runs without contamination from actual reward feedback and learning during the session (Adams, 1982; Balleine and Dickinson, 1998). We found that in control rats, which had received virus injection but lacked effective illumination, the over-training protocol induced a maze running habit: in the probe test, the over-trained rats ran readily to the devalued goal when so instructed, just as they had before the devaluation, and just as much as they ran to the still valued goal (Fig. 2, black line, compare last over-training day before devaluation and probe day). Thus, these rats were running the maze as though they were no longer sensitive to current outcome value, but instead, were running by habit.

Rats with illumination of eNpHR3.0-expressing IL neurons during the maze runs behaved very differently during the probe sessions (Fig. 2, probe day, compare red line to black line). Every rat ran in a goal-directed manner: they reduced and mostly stopped running to the devalued goal (Fig. 2, red line, compare last over-training and probe days). Instead, they began running away from it, to the non-devalued goal arm (a behavior that was never rewarded). Thus, on-line perturbation of IL activity during maze runs blocked the acquired habit of these overtrained rats. It was as though the perturbation of the IL cortex allowed them to run with a more forward-looking, purposeful approach in which they were capable of evaluating the outcomes of the runs and adjusting performance accordingly (Dickinson and Balleine, 2009; Killcross and Coutureau, 2003). Because there were no rewards at all in these probe trials, the rats had to be using an altered internal strategy to guide their runs.

This finding suggested the possibility that the IL cortex can control habits on-line during the performance of the habit. If so, the IL cortex could not only be generally necessary for habit expression (Coutureau and Killcross, 2003; Hitchcott et al., 2007; Killcross and Coutureau, 2003), but specifically could be necessary during on-line performance. Disrupting IL activity within single maze runs in this way could have cascading effects over neuropsychological processes occurring after goal arrival, or even after the session. We saw, however, no consistent rebound in neuronal activity after termination of the illumination, and no significant prolonged effects in the IL cortex itself. Moreover, in rare instances, we omitted the light during individual runs and found that the rats returned to habitual behavior. Thus, while the behavioral effects of IL perturbation could have involved an avalanche of changes triggered by the short disruption of spike activity, the key finding is that these brief, 2–3 sec periods on light delivery were enough to nullify the habitual behavior in individual runs.

## 5. IL perturbation blocks a replacement habit and restores an original habit

A major advantage of the optogenetics method for this work is that interventions are repeatable, in addition to being brief. We adopted such repetition strategies over several subsequent weeks of maze training, in which rewards were again provided for correct performance (one being devalued, one still valued). During these post-probe reward sessions, all rats sampled the devalued goal when instructed to go there a few times, and also ran to it on occasion without drinking any of it, and then rapidly stopped running to the devalued goal (Fig. 2, post-probe stages 1 onward). They instead developed a new strategy of running the wrong-way to the non-devalued side when cued to the devalued goal, despite there being no reward available for those incorrect runs. These wrong-way runs grew more and more frequent over time, despite never yielding reward. The rats developed what appeared to be a new replacement habit, one of always running to the non-devalued goal no matter what the instruction cue indicated (Fig. 2, right cartoon insert). This second habit appeared to form relatively quickly, which was likely due to the familiarity of the running route and task rules. We then asked whether further IL perturbation would affect the new wrong-way running behavior. We initially looked at the first few days after devaluation, when the rats were beginning to adjust their behavior to tasting the devalued reward, to see whether IL perturbation would facilitate this new learning and behavioral flexibility. It did not. The controls and IL-perturbation rats behaved quite similarly, avoiding the devalued goal on most trials once they could taste it (Fig. 2, post-probe stage 1), consistent with the strong potency of taste aversion conditioning on appetitive behavior (Adams, 1982; Garcia and Ervin, 1968; Holland and Straub, 1979). This result suggested either that a lower limit had been reached in the runs to the devalued goal, which IL perturbation could not decrease further, or that the IL cortex was no longer engaged in guiding behavior in this period of new learning (i.e., when rats were no longer running habitually). Additional optogenetic

perturbation tests conducted within 6 days similarly had no detectable effect on the rats' behavior.

We then applied a perturbation of the IL cortex about two weeks after devaluation, when the wrong-way runs had grown routine (Fig. 2, post-probe stage 6). We reasoned that, on the one hand, the original habit had probably been 'broken' by this point, so that further perturbation might have no effect just as it had failed to make much difference during initial post-devaluation days. On the other hand, if the rats were developing a new habit, the IL cortex might also be promoting this new habit on-line, just as it had promoted the first habit. What we found supported the second possibility, but with a surprising twist. When we disrupted the IL cortex at this later stage, the wrong-way runs decreased markedly, suggesting that the late IL manipulation did block this new habitual routine. Remarkably, when these wrong-way runs were blocked, the rats suddenly behaved as they had during over-training: they ran correctly when instructed to each goal, and when they arrived they drank each reward (Fig. 2, red line). Thus, the same IL intervention that had suppressed the initial habit earlier now, instead, appeared to reinstate it, in the same rats, at the same IL sites, and with the same light delivery protocols (Fig. 2, red line, compare last over-training day to stage 6). This effect was sudden and stepwise in each animal. This effect of the IL intervention appeared to be long-lasting (Fig. 2, red line, stage 7 onward). Light exposure amounting to ca. 2 min during a task session resulted in at least days-long reinstatement of the habit, suggesting that neuroplasticity occurring in relation to brief IL perturbation can produce a lasting change in behavior when silencing is removed.

In additional reward-drinking tests (Smith et al., 2012), we found no evidence for change in general motivation after the IL perturbation, consistent with former work modulating IL activity by drug microinjection (Hitchcott et al., 2007). These tests were conducted in the rats' home-cages on days between post-probe maze training days, and involved one session with equivalent IL illumination (3-sec-on/10-sec-off cycles over 45 min) and one with no illumination. The rats consumed the same amount of the reward liquid whether the laser stimulation was on or off, both just after devaluation and later, when the in-maze habit reinstatement had occurred. Moreover, although rats had begun to drink more of the devalued reward at home after the many days since devaluation, regardless of light delivery, it was not as though the in-maze reinstatement that occurred at this time reflected a close tracking of the reward value, as if the devalued reward had grown much less aversive. In contrast to control rats, which continued to avoid the devalued goal on the maze throughout this entire time despite a few occasions each day in which the devalued reward was tasted (drinking far less on the maze than in the home-cage), the IL-perturbation group suddenly dropped this wrong-way running behavior upon IL inhibition and ran readily to the devalued goal. The loss of some at-home aversion after extended post-devaluation training may have aided the capacity for the late IL perturbation to reinstate the initial habit, but this falls short in accounting for the full effect (Smith et al., 2012). In addition, crucially, performance on trials in which the rat was instructed to the non-devalued goal was always accurate and unaffected by IL perturbation throughout the experiment (Smith et al., 2012). This set of findings indicated that the rats had formed a new habitual routine during the post-devaluation training period, and that once this was formed, further IL perturbation blocked this routine also and uncovered the initial habit that was somehow being repressed.

The strikingly rapid time-course with which IL perturbation could influence behavior provided further support for this interpretation (Fig. 3). In the initial habit-blockade probe session (Fig. 3A), and in the later habit-reinstatement session (Fig. 3B), the effects of light treatment on behavior were nearly immediate. Rats changed their behavior within a few trials, and occasionally on the very first trial, amounting to only seconds of IL perturbation. This rapidity was clear on the habit reinstatement day (Fig. 3C, right) relative to that of the



control group on the same day (Fig. 3C, left) or that of the IL-perturbation group's performance on the previous day (Fig. 3C, middle). This finding strongly favors the interpretation that their behavior during IL illumination reflected a known course of action (i.e., a stored set of response rules) rather than new in-session learning through trial-and-error. We suggest that initially, IL perturbation uncovered the prior stored strategy of purposeful running, with guidance by outcome value (as suggested by IL lesion results (Killcross and Coutureau, 2003)). Later when wrong-way runs were established, IL perturbation uncovered the cached strategy prior to that, which was to run habitually to both goals.

## 6. Advantages of the optogenetic approach

We have used this example to highlight the advantages of optogenetics for giving a new level of discreteness to interventions aimed at identifying circuit functions in the nervous system. These have broad applicability:

### Temporal resolution

Optogenetic manipulations allowed us to manipulate IL neural activity in a time-locked fashion only during habit performance. Chemical inactivations produce effects that begin prior to task onset, and that continue through intertrial intervals and probably also post-task time. The optogenetic strategy pinpointed the manipulation to the time of performance only, ruling out contributions from pre-trial planning time, reward consumption time, or post-trial processing time. Beyond the aims of our own experiment, it will be possible to evaluate causal relationships between neuronal activity and habits at even finer timescales (e.g., only during a segment of a maze run, only during a lever press, etc.), as well as to change the manipulation parameters across individual trials.

### Repeatability

There is no fundamental barrier to repeating optogenetic manipulations over many test days. This feature is nearly unprecedented amongst prior methods of neural intervention, including drug microinjection strategies, which are typically limited to a half dozen or so due to accrued tissue damage. Thus, we could test the effect of IL perturbation on maze running at many points in time within single animals. Future work might further exploit this repeatability to examine the role of brain regions over even longer stretches of habit learning time.

### Spatial resolution

Gene-based targeting strategies in general allow for a remarkable level of specificity in controlling subclasses of neurons. Using viral mediated gene transfer, opsins can be introduced into brain regions in a cell-type specific manner. In our work, we used a construct containing the CaMKII $\alpha$  promoter targeted the opsin preferentially to excitatory IL neurons. By comparison, chemical agents, such as GABA agonists, would influence a broader spectrum of cell types. In the future, habit research will surely capitalize on the many other possibilities for cell-type specific manipulations, including through the use of cre-dependant expression technology. Pathway-specificity is also achievable with viral mediated gene transfer approaches, allowing researchers to address circuit-level questions that have been essentially impossible until now. For example, some optogenetic viral constructs can lead to opsin expression in the axons and terminals of infected cells, in addition to the cell bodies, making it an option to shine light directly on the terminals to perturb the activity of neurons embedded in a specific pathway of interest (Brown et al., 2012; Pascoli et al., 2012; Stuber et al., 2011; Tye et al., 2011; Warden et al., 2012).

## Electrophysiological readout

It is possible to evaluate drug microinjection-induced changes in neuronal spiking at the injection site in the behaving animal, but in practice this is difficult and often tenuous (e.g., due to distortion of recorded spike waveform shape by injections, instability of tracking single neurons to establish an onset/offset time-course, etc.). The use of light makes monitoring changes in physiological activity more accessible. As a result, it will be feasible to not only establish a behavioral effect of optogenetic interventions, but to also establish the change of neuronal activity that it correlates with and to identify recorded neurons as belonging to a certain subclass that was targeted by the viral construct (Brown et al., 2012; Cohen et al., 2012; Kravitz et al., 2013). There are a variety of procedures to integrate fiberoptic light delivery with electrical recordings, so that readouts of light-evoked changes in neural activity can be made without the researcher having to interfere with on-going animal behavior (Anikeeva et al., 2011; Kravitz et al., 2013). We chose the route of customizing off-the-shelf recording head-stages to incorporate fiberoptic light delivery guides alongside tetrode implants, and thereby could identify changes in spike activity of IL neurons that occurred during light delivery (Smith et al., 2012).

## Localization of perturbed regions

It is a common practice to incorporate fluorescent markers in viral constructs for later detection in brain tissue. In some constructs, such as the one we used, the fluorophore (eYFP) is genetically fused to the opsin. Thus, when the fluorophore is detected in a population of cells using natural fluorescence or antibody stains, one can be reasonably confident that the opsin was present as well. In this way, the cell population that was potentially impacted by light can be imaged. With traditional chemical injections, by contrast, visualization options are far more limited (e.g., by using fluorescent compounds or autoradiography, which do not label cells, or using IEGs or similar markers that generally cannot distinguish directly and indirectly affected cells). Also, as noted, many of the viral constructs can be used to detect axons and synaptic targets of the infected cells, thus providing data on anatomical connectivity essentially 'for free'. This allowed us to confirm that the IL region we targeted in our feature experiments projected to medial, but not lateral, striatum (Smith et al., 2012).

There are of course relative limitations to optogenetic methods even for loss of function studies, making it more of a companion tool for traditional methods rather than a replacement. For example, lesions, and arguably injections, are valuable if large volumes of tissue must be covered (though, new light delivery methods and red-shifted opsins, or opsins with especially large photocurrents, are being engineered to perturb large regions of brain; pharmacogenetic approaches also provide a solution to this issue). Also, injected pharmacologic compounds can uniquely target specific receptor subtypes to address questions about their role in the temporary control of behavior. Nevertheless, considering the advantages noted above and in comprehensive reviews on the technique (Bernstein and Boyden, 2011; Fenno et al., 2011; Tye and Deisseroth, 2012), an optogenetic approach was ideally suited to address the question of on-line control over habits by the neocortex. Finally, although in the field now opsins are described as being 'excitatory' or 'inhibitory', much accumulating evidence, along with our own, shows that the effects are mixed when viewed across the cell population of a targeted area. Microcircuit connectivity as well as long-distance interactions among neurons (and glia) likely account for these effects.

## 7. Questions for future research on optogenetic control of habits

Our findings in these first optogenetic experiments on habits raise focused questions for further experimental work. For instance, it appeared that the memory of the original habit

had been suppressed but not abolished during the post-devaluation phase, supporting an idea dating back to Pavlov that learned responses are often overridden and replaced, but not lost (Pavlov, 1927; Rescorla, 1996). It was as though there were layers of response tendencies being built up, and that optogenetically shutting off the IL cortex peeled away the acting layer to expose the prior one. In other research fields, the IL cortex has been implicated in regulating the extinction of conditioned responses (Peters et al., 2009; Rhodes and Killcross, 2004), in maintaining a new shift in performance strategies on maze tasks (Ragozzino, 2007; Rich and Shapiro, 2009), and in the ability to withhold behavioral responses or to 'wait' (Chudasama et al., 2003; Ghazizadeh et al., 2012). Notwithstanding the many task differences across these studies, one common function of the IL cortex appears to be to maintain a new behavioral strategy atop an old one; without an IL cortex, the acting strategy is gone leaving the old prepotent one to be expressed (Coutureau and Killcross, 2003). The IL cortex might therefore maintain new response strategies that compete with old ones, even old habits. This working hypothesis suggests that the IL cortex operates at a supervisory or executive level in the selection specifically of *newly* acquired habit strategies (Smith et al., 2012). Our findings are also congruent with models of habitual behavior in suggesting that the detailed habit memory likely did not reside in the IL cortex, because the habit was reinstated when IL cortex was taken off-line. Where and how the details of habits are learned, stored when suppressed, and accessed by this IL-associated executive system are open and fascinating questions. One clear possibility is that the DLS contributes to this function, but this idea must be tested.

The multitude of behavioral functions linked to the IL cortex raises the possibility that the nature of the optogenetically evoked changes in behavior could be resolved in even more detail. It is unlikely that the IL perturbation produced generalized effects on reward, motivation, or performance that masqueraded as habit changes, because home-cage drinking behavior and runs to the non-devalued goal were unaffected. It also appeared to produce changes in maze running that were too rapid to be accounted for by new trial-and-error learning. Still, even accepting the immediacy and context-specificity of the laser effect, as well as the opposing effects on the probe day as compared to the later day (post-probe stage 6), the maze-running changes that we observed could be a consequence of several distinct processes contributing to habit performance of the sort we tested, which might be studied independently (e.g., maze-specific changes in outcome-representation, cue triggered motivation, action selection, generalization of the taste aversion, etc.). Similarly, it will be important to extend these results to non-maze task environments. Tasks that involve multiple cues, decisions, and responses can prevent the formation of a devaluation-insensitive habitual response (Colwill and Rescorla, 1985; Colwill and Triola, 2002). The clear formation of habitual behavior on the T-maze is surprising in this sense, and might reflect the strong navigational component and/or the extension of task stimuli and responses over space and away from the goal location. Whether these habit reinstatement effects of IL perturbation are unique to our particular task environment is an open question, though the effect of IL inhibition on blocking an initially acquired habit has been observed using several tasks and devaluation methods (Coutureau and Killcross, 2003; Hitchcott et al., 2007; Smith et al., 2012).

In these first experiments, we have also only begun to tap the spatiotemporal precision that optogenetic tools can provide. It remains unknown whether habits of this sort can be turned off trial-by-trial, or if once they are turned off they are off for good. Support for the second possibility comes from our finding that the behavioral effects observed during runs in which the IL cortex was inhibited seemed to persist over subsequent days that lacked silencing. Such questions can best be answered by varying the timing of inhibition, for example alternating trials with it and without it. Also unknown is the time-course by which IL cortex operates to promote habit emergence. We began to pursue this question in the habit

reinstatement effect by varying when after devaluation we disrupted IL cortex. This strategy suggested an inflection rather than gradual slope of reinstatement process by which suppressed habits can re-expressed. This finding indicates that habits might normally be reinstated this way after a period of suppression (i.e., reinstated in nearly full force, as we know intuitively to be true of some habits).

The anatomical route by which the IL cortex exerts its influence over habits remains essentially unresolved. In several regions receiving IL output, we and others have observed activity that appears to be very closely related to potential roles in flexibility and outcome sensitivity. For example, during over-training, we have found a decrease in activity in the prelimbic cortex as a habit emerged (Smith and Graybiel, in submission). Also, in similar T-maze tasks, we have found that activity in the DMS at the cue and decision period strengthens with training, but weakens with over-training (Thorn et al., 2010). Similarly, along the same time-course, ventral striatal activity emerges at the initial task cue and weakens to the goal, while goal-related beta oscillations increase (Atallah et al., 2010; Howe et al., 2011). These patterns add to growing evidence that a waning or restructuring of activity in networks promoting goal-directed behavior and outcome/reward processing might occur as habits emerge (Balleine and O'Doherty, 2010; Graybiel, 2008; Yin et al., 2009). Orchestrating this striatal plasticity is a potential function of the IL cortex to probe in future work, particularly by evaluating how changes in IL activity that affect habits modulate motivational and reward-related signals in its output striatum, and other targets, as is being examined in related fields (Ghazizadeh et al., 2012; Pascoli et al., 2012; Peters et al., 2009).

There is similarly great potential for harnessing optogenetic methods to examine striatal mechanisms underlying habit learning. This potential is exemplified by the recent work of several groups on striatum roles in movement and reward learning, such as highlighted in this special *Brain Research* issue and elsewhere (Kreitzer and Berke, 2011; Lobo et al., 2012; Stuber et al., 2012). The striatum contains subtypes of interneurons, as well as projection neurons, that are embedded in distinct pathways including the direct striatonigral and indirect striatopallidal pathways. Until recently, it was not possible to target these cell types independently and transiently during behavior. Now, for example, cre-dependent expression technology has been used in the dorsal striatum to show that mice will favor an action that leads to optogenetic stimulation specifically of dopamine D1 receptor-containing neurons (mainly direct pathway), suggesting activity of these neurons can increase motivated behavior or is intrinsically rewarding (Kravitz et al., 2012). By contrast, mice will favor an action that does not lead to stimulation of D2-expressing (mainly indirect pathway) neurons, suggesting that activity of these neurons produces motivation to avoid stimuli or select alternate behaviors, or that it is intrinsically aversive. Similarly, by targeting these different striatal pathways using viral constructs coding for a synthetic receptor (hM(4)D) and injecting a synthetic ligand (clozapine-N-oxide), it has been established that inhibition of the direct pathway can augment the psychomotor sensitizing effects of stimulant drugs, while inhibition of the indirect pathway impairs it (Ferguson et al., 2011). Related optogenetic work in ventral striatal circuits has begun to demonstrate distinct microcircuits for stimulus preference and aversion (Brown et al., 2012; Lobo et al., 2010; Witten et al., 2010) and motor control (Kravitz et al., 2010). Given evidence that habit learning is encoded in the activity of subpopulations of neurons (Barnes et al., 2005; Jog et al., 1999), and evidence that genetic knockout of the adenosine A2A receptor in striatum (expressed in indirect pathway neurons) blocks habit expression (Yu et al., 2009), there is reason to suspect that a similar cell-type-specific optogenetic strategy will lead to significant progress in understanding striatal control over habitual behaviors.

## 8. Extreme habits and disorders involving a flexibility/fixity balance

Dysfunction in cortico-basal ganglia and cortico-limbic circuitry has been suggested to result in excessive variability or fixity of thoughts and actions in neuropsychiatric disorders, including addiction, obsessive-compulsive spectrum disorders, and mood disorders (Albin and Mink, 2006; Belin et al., 2009; Graybiel and Rauch, 2000; Holtzheimer and Mayberg, 2011; Leckman and Riddle, 2000; Redish et al., 2008). Optogenetic approaches are proving to be effective research tools in these domains as well (Lobo et al., 2012; Stefanik et al., 2012; Stuber et al., 2012; Tye and Deisseroth, 2012; Witten et al., 2010). Addiction, for example, appears to involve in part a tip in the balance between the flexibility-promoting and habit-promoting networks discussed here, leading to exaggerated motivation, loss of outcome-sensitivity, and failure of cortical control over response impulses (Belin et al., 2009; Hogarth et al., 2012; Hyman et al., 2006; Jedynak et al., 2007; Kalivas and Volkow, 2005; Schoenbaum and Setlow, 2005). The IL cortex is one of the several regions implicated in these effects (Bossert et al., 2011; Peters et al., 2009; Porrino and Lyons, 2000).

Related to addiction, optogenetic intervention has now been used to target IL projections to the nucleus accumbens (Pascoli et al., 2012). This study showed that, normally, injection of cocaine in mice resulted later in potentiation of corticostriatal synapses on D1-expressing neurons and a parallel sensitized motor response to cocaine exposure. However, if optical stimulation of IL-originating fibers in the accumbens shell (which depotentiated the synapses) was delivered before the test, the drug-evoked neuroplasticity was gone and animals failed to exhibit motor sensitization. This work provides evidence for the role of this specific pathway in regulating a key feature of long-term neuro-behavioral adaptations to drugs, and highlights the utility of such fine-scale optogenetic manipulations in resolving brain mechanisms underlying excessive behavior. The link to our own work, reviewed above, suggests that there may not be so great a distance as has been thought between the control mechanisms for normal habit making and the extreme habits that themselves are likely related to neuropsychiatric disorders.

## 9. Conclusion

To our great benefit, work on habit formation has progressed from being framed mainly by the early psychological constructs of behavior as being almost entirely reflexive to a far more specific level of definition that makes habitual behavior a tractable subject for neurobiological research. There is a sense now that we can use these foundations to begin resolving how the multitude of variables described above arise and interact with one another. On the one hand, the picture we are left with is a familiar one: habits are complex and probably involve dynamic changes in brain activity across multiple functional circuits, at multiple points in time (Belin et al., 2009; Graybiel, 2008; Hogarth et al., 2012; Redish et al., 2008; Robinson and Berridge, 2008; Seger and Spiering, 2011; Wood and Neal, 2007; Yin and Knowlton, 2006). But on the other hand, the work of many groups and the melding of multiple technical approaches have begun to establish what these dynamic changes are and where they occur, as well as to advance the conceptual frameworks used to understand them. Questions left unanswerable by traditional tools, specifically concerning the causal influence of genetically defined cell types, pathways, and millisecond-scale patterns of neural activity over habitual behaviors, can now be addressed. Our work reviewed here provides just one small step in this direction, but hopefully gives the impression of the great potential for this kind of approach.

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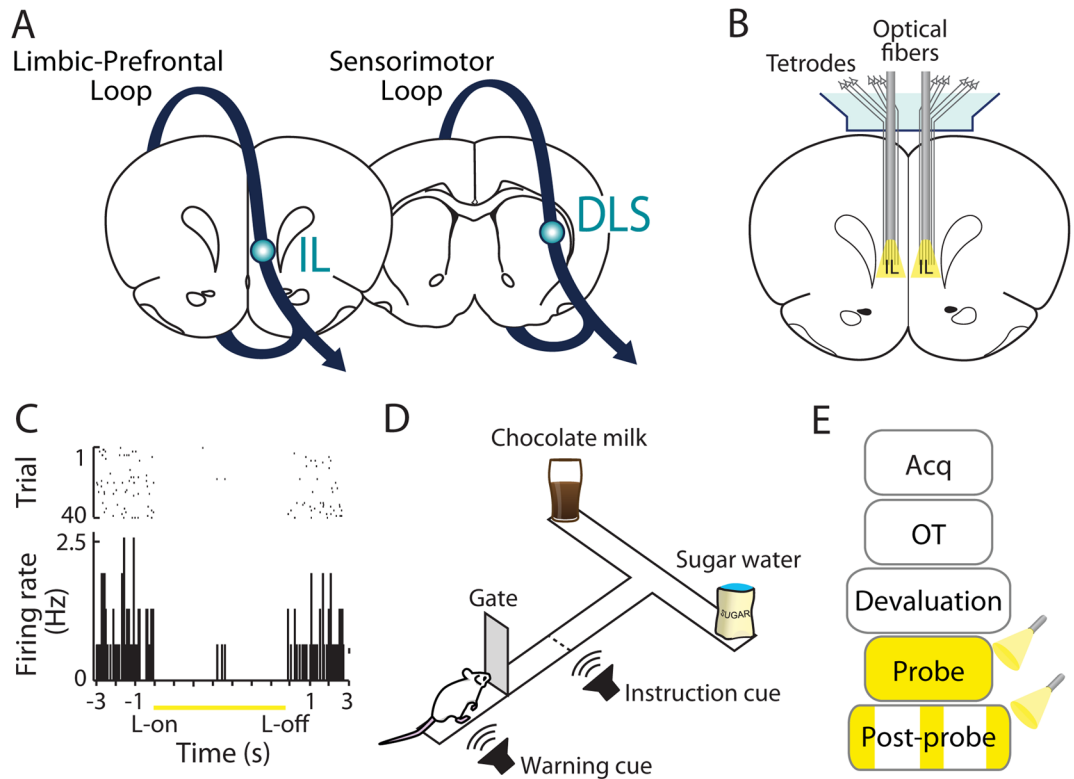
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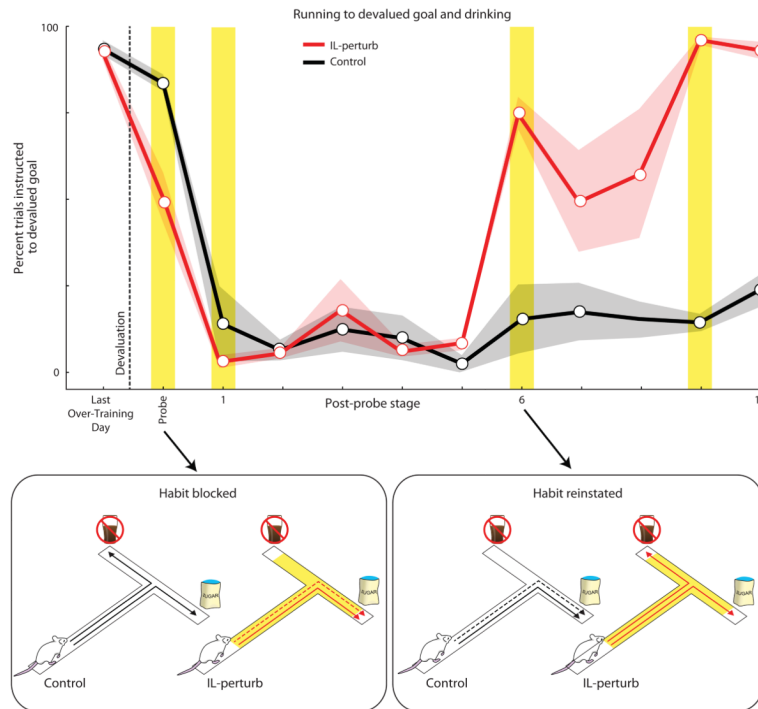


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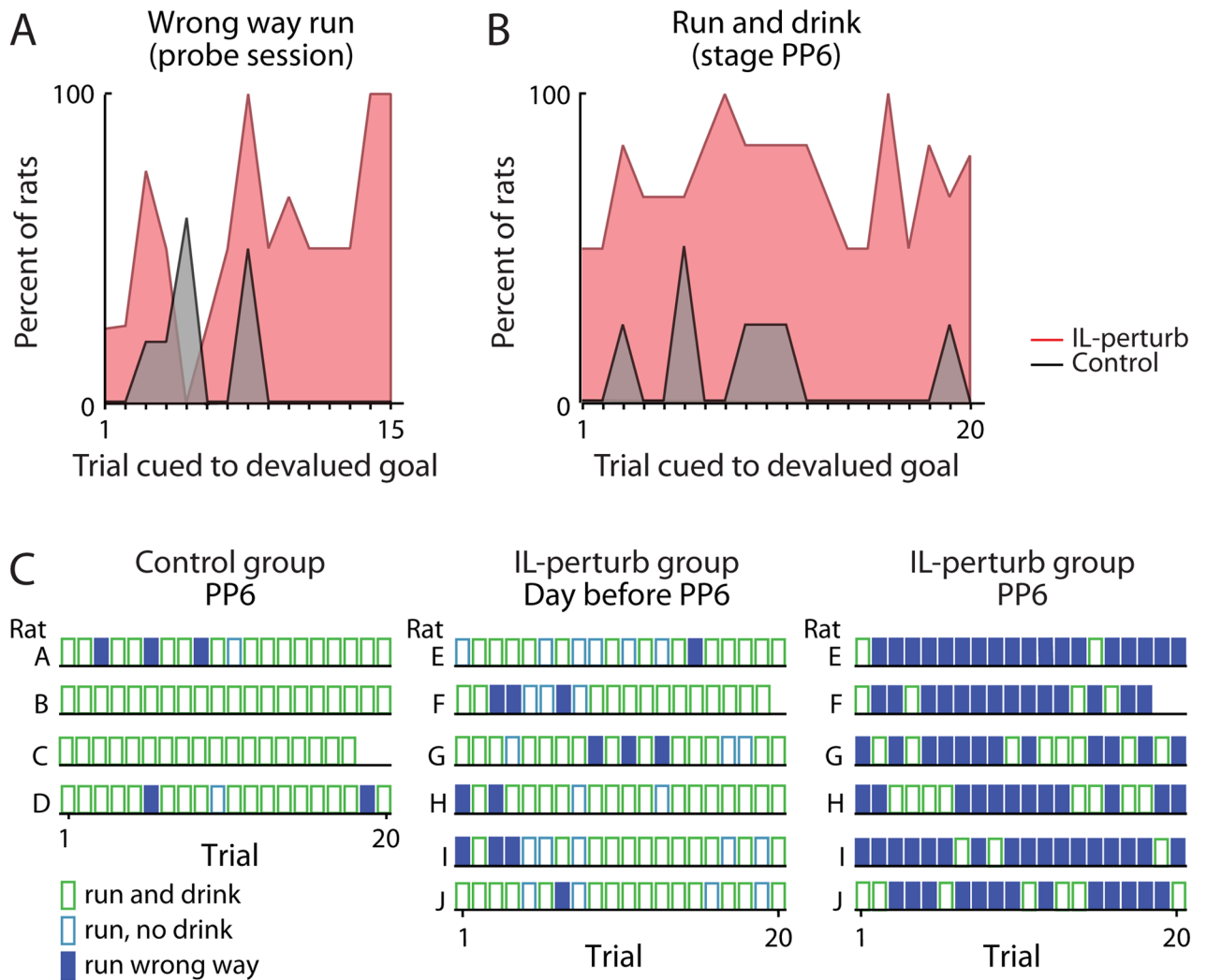
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**Fig. 1.**

Protocol for habit learning and IL perturbation. (A) Diagram of coronal brain sections showing habit networks related to the infralimbic (IL) cortex and dorsolateral striatum (DLS). (B) Diagram of modified recording head-stage containing tetrodes and optical fibers targeting IL cortex. (C) Raster plot and histogram of a representative IL unit, showing suppression of spike activity time-locked to yellow light delivery. (D) T-maze task. After a warning cue and gate opening, the rat traverses a runway, and then hears one of two cues signaling whether reward will be available at left or right goal-arms on that trial. One goal arm is paired with chocolate milk, and the other with sucrose solution (counterbalanced between animals, but fixed within animals). (E) Task timeline, top to bottom: task acquisition (Acq), over-training (OT), reward devaluation, unrewarded probe test (with IL illumination), and post-probe training days (with periodic IL illumination). Modified from (Smith et al., 2012).



**Fig. 2.** Habit blockade and reinstatement timeline. Percentage of trials cued to the devalued reward in which control rats (black) or rats with IL-perturbation (red) ran there and drank it, is shown for the day prior to devaluation, the probe day after (only runs shown), and the post-probe (PP) rewarded stages. Yellow bars denote days of IL perturbation. Below, cartoon depictions of the habit blockade on the probe day (left) and habit reinstatement later on PP6 (right). Normally, control rats acted habitual on the probe day, as they continued to run to the devalued goal, whereas rats with IL inhibition reduced runs by about 50%. Further IL inhibition on PP1 had no effect. Both groups rarely ran to the devalued reward and drank over post-probe days (PP1-5 = 2 weeks), instead running the wrong-way to the non-devalued goal. However, when IL cortex was inhibited again (PP6), rats ran back to the devalued goal and drank. This effect remained apparent in subsequent days, and was bolstered when IL cortex was inhibited again (PP9). Control rats maintained a very low level of pursuing the devalued goal throughout this time. We include here new statistics to confirm these observations, using ANOVA and Tukey-correct pairwise comparisons to analyze key training stages (last overtraining day, probe day, stage PP6). For the IL-perturb group, a significant day effect was found ( $F_{2,14} = 19.34$ ,  $P < 0.001$ ). Runs to the devalued goal on the probe day were fewer compared to the last overtraining day or PP6 (each  $P < 0.01$ ), while there was no difference between the last overtraining day and PP6 ( $P = 0.087$ ). Thus, there were significantly more runs to the devalued goal on laser day PP6 than on the laser probe day (i.e., dissimilar to the replacement habit), roughly equal to the number of those runs on the last over-training day (i.e., similar to the original habit). Modified from (Smith et al., 2012).



**Fig. 3.** Immediacy of IL perturbation effects on behavior. (A) Percent of IL-perturb (red) and control (black) rats that performed a wrong way run during single trials instructed to devalued goal in the probe test. 100% = all rats ran the wrong way on that trial; 0% = no rats ran the wrong way (i.e., all ran to devalued goal). Rats with IL inhibition ran the wrong-way early, on the first trial for some rats, and then kept up the wrong-way runs over the full session. (B) Trial plot of the “reinstatement” day (post-probe stage 6 from Fig. 2) showing percent of rats that ran correctly to the devalued goal and drank. Again, the effect was rapid: rats ran back to the devalued goal on the first few trials, and continued running there for the session. (C) Left: behavior of each control rat, trial-by-trial, on the reinstatement day when instructed to the devalued goal; middle: behavior of each IL-perturb rat on the day before IL inhibition and reinstatement; right: each IL-perturb rat on the day of reinstatement during IL inhibition. A robust number of runs to the devalued goal (blue) were observed only during light delivery in the halorhodopsin treated rats. The rats ran there early in the session, and then kept pursuing it in long bouts of repeat runs and drinks. By contrast, control rats, and the IL-perturb rats on the day before, sampled the devalued goal only sporadically. Modified from (Smith et al., 2012).