and function in several directions. This work pulls vinculin into a central position in the model of mechanotransduction at sites of adhesion. Tension-dependent switching of vinculin is a master step in the stabilization or destabilization of focal adhesions, and cycles of vinculin switching determine focal adhesion dynamics during cell migration.

It will be important to investigate whether vinculin functions similarly at cadherin-based junctions. This mechanism may also function at sub-focal adhesion scales because recent work by the Waterman lab indicates that tension varies even within focal adhesions [16] and work by the Geiger lab has shown that vinculin and paxillin stability are increased in the distal parts of focal adhesions that Waterman identified as high-tension regions [17].

With the current high standards of imaging and biophysical experimentation we are coming close to understanding focal adhesions and their mechanotransduction at the molecular level. The implications of all of this for development and disease, however, remain somewhat obscure. It is clear that there are consequences for cell migration, but mechanical properties of the ECM also regulate morphogenesis and differentiation in development [18] and proliferation and de-differentiation in tumor progression [19,20]. It will be interesting to use the acquired molecular knowledge to assess the importance of vinculin-dependent mechanotransduction in these processes.

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Motivated Action: New Light on Prefrontal-Neuromodulatory Circuits

A new study has used optogenetic methods to stimulate prefrontal-brainstem neuromodulatory pathways while animals face environmental stressors, the results providing further compelling evidence that prefrontal control of neuromodulatory function can have a dramatic effect on motivated behavior.

Amitai Shenhav[1] and Matthew M. Botvinick[1]

For many years, research on prefrontal cortex was characterized by a focus on 'cold' rule-driven behaviors. More recently, prefrontal cortex research has been heating up, attending increasingly to issues involving motivation and reward. Numerous regions within prefrontal cortex — alongside subcortical structures more classically associated with affect and motivation — are now considered to be critical for linking motivation to behavior. Still more recently, there has been a trend toward better understanding how this link arises from interactions within and across cortical-subcortical *circuits* [1,2]. A new paper by Warden *et al.* [3] is an important advance in this direction.

Through a combination of innovative techniques, Warden *et al.* [3] were able to explore how medial prefrontal

cortex (mPFC) interacts with two subcortical structures, the dorsal raphe nucleus (DRN) and lateral habenula (LHb), in reacting to environmental stressors. Specifically, they placed rats in an inescapable and narrow pool of water — a classic assay for depression-like behavior known as the forced swim test — and recorded from mPFC while examining how much time the rat spent swimming in place versus passively floating (the latter often being interpreted as a signature of depression, 'giving up'). They show that a set of neurons in mPFC anticipate and persist in firing throughout periods where the rat continues to swim.

Through optogenetic stimulation, Warden *et al.* [3] were able to show further that the amount of time the rat spends engaging in this behavior is specifically influenced by projections from mPFC to DRN. Stimulation of mPFC alone does not influence mobility, but stimulating mPFC–DRN projections increases mobility. Importantly, stimulating the mPFC–DRN projection does not simply increase mobility whatever the environment. The authors found that mobility was not affected when this pathway was stimulated in a more motivationally neutral environment. They did find a more general increase in mobility across both settings when stimulating DRN directly. Interestingly, they found that stimulating mPFC's projection to another subcortical structure, the LHb, produced the opposite behavioral effect: stimulating the mPFC–LHb pathway *decreased* the rat's mobility during the forced swim test.

The Warden *et al.* [3] study adds to a growing literature on the role of mPFC in motivation and decision making. Research has indicated, for instance, that regions of mPFC encode the rewards associated with available actions, and play a role in licensing the expenditure of effort in pursuit of those rewards [2,4,5]. Moreover, lesions to the prelimbic region of rodent mPFC have been found to result in less goal-directed, more habit-bound behavior [1,6]. Given such findings, it is tempting to interpret the new observations from Warden *et al.* [3] in terms of both goal-directed and effort-based decision making, by assuming that rats in the forced swim test are actively considering the costs and benefits of action, and in some cases deeming the effortful swimming behavior to be 'worth it.' While this fits broadly with the reported findings, two caveats arise. First, the region of mPFC most commonly implicated in effort-based decision-making (anterior cingulate cortex [4]) lies outside the area probed in the current study, which instead spanned prelimbic and infralimbic cortices. An effort-mobilization account also runs up against another finding from the study: while mPFC neurons tended to be more active during swimming than immobility in the forced swim test, they *also* tended to be highly active during a period prior to the forced swim test, when the animal occupied a familiar cage and remained largely inactive.

The second caveat pertains to the question of whether swimming in the forced swim test should be viewed as a 'goal-directed' behavior [1]. Prelimbic cortex has been argued to share some functional analogs with primate dorsolateral prefrontal cortex [7], a region implicated in the goal-directed overriding of automatic or default responses. This fits intuitively with the apparent involvement of mPFC in the Warden *et al.* [3] study, in facilitating an active rather than passive response to the forced swim test. But it is hard to see animals' almost total inactivity prior to the forced swim test — which was accompanied by robust mPFC activity — as a controlled or goal-directed behavior. In considering the resulting quandary, it may be worth considering the possibility that swimming may be not a controlled, instrumental response to immersion, but instead an automatic or Pavlovian [8] response, and that mPFC is favoring Pavlovian over instrumental control. If this seems to strain against the traditionally assumed role of prefrontal cortex in overriding automatic behavior, it may be worth considering that part of the mPFC region studied by Warden *et al.* [3] falls within a set of areas recently proposed to parallel the primate 'default mode' network, a network whose activity typically *anti-correlates* with the exertion of top-down behavioral control [9,10].

This perspective also offers a clearer picture of how the Warden *et al.* [3] study might be understood within the context of earlier research linking mPFC with serotonergic function. Previous studies have suggested that mPFC interacts with the DRN to shape behaviors naturally elicited by environmental stressors. In particular,

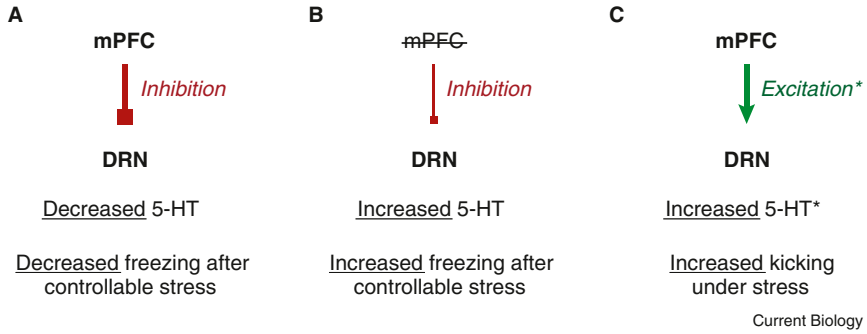

Figure 1. Relationship of mPFC, DRN, and serotonin (5-HT) levels to behavior under stress.

The relationship between DRN serotonin (5-HT) release and behavior under stress suggests a potential role in licensing automatic or Pavlovian behaviors. (A,B) Amat and colleagues [12] showed that DRN release of 5-HT can be associated with response inhibition (freezing). They gave rats a series of controllable (escapable) or uncontrollable (inescapable) shocks, and the rats then received a few shocks in a new context. (A) Relative to animals receiving uncontrollable shocks, animals with intact mPFC inhibited DRN 5-HT release to controllable shocks, and froze less in the new context. (B) Lesioning mPFC reversed both of these effects: lesioned rats still learned how to terminate (control) the shocks, but failed to inhibit DRN 5-HT and froze just as much as rats who had been exposed to uncontrollable shocks. (C) Studies by Warden *et al*. [3] and Hamani *et al*. [13] associate DRN serotonin release with the activation of an arguably automatic or Pavlovian response (kicking). They show that stimulating mPFC directly [13] or specifically stimulating mPFC-DRN projections [3] leads to increased mobility in the forced swim test. *Note that the excitatory influence of mPFC on overall DRN 5-HT release is speculative but inferred from Hamani *et al*.'s [13] finding that forced swim test behavioral changes depended on increasing 5-HT levels. Warden *et al*. [3] do not directly test whether mPFC stimulation has an overall excitatory or inhibitory influence on DRN 5-HT release.

the DRN's release of serotonin is known to increase in the context of uncontrollable stressors, such as electric shocks that an animal has no way to avoid [11]. This typically leads to generalized increases in freezing behavior when the animal is placed in an unrelated context and experiences a few shocks there. When an animal is instead given control over when the shocks terminate, an intact mPFC causes the DRN to release less serotonin, and decreases subsequent freezing behavior [12]. Lesions to mPFC result in greater serotonin release and in freezing behaviors, as if the shocks had been uncontrollable. Placed alongside the new study by Warden *et al.* [3], and a recent study that similarly showed stimulation of mPFC leading to increased forced swim test swimming and increased serotonin levels [13], these findings can be collectively understood in terms of a link between serotonin and the 'licensing' of pre-programmed or Pavlovian responses to aversive situations (Figure 1). Whether expressed as freezing in the context of shock, or swimming in response to immersion, decreasing DRN serotonin release puts the brakes on such behavior, and increasing DRN serotonin release lifts those brakes.

This speculative gambit fits broadly with recent computational work on serotonin from Boureau and Dayan [14], who have proposed that serotonin may signal expected punishment, facilitating associated Pavlovian responses (typically, but not necessarily, taking the form of behavioral inhibition). However, it is important to note that the gambit rides on the assumption that stimulation of the mPFC–DRN pathway stimulates serotonin release. It is easy to read Warden *et al.* [3] as implying such an excitatory effect, and previous work by Hamani *et al.* [13] offers some corroboration that increased forced swim test swimming can result from increased serotonin levels. Given that LHb carries primarily inhibitory projections to DRN [15], the opposite behavioral effect of mPFC–LHb stimulation could likewise be seen as an opponent influence on serotonin release. However, caution is warranted on both counts. There is evidence that the preponderance of projections from these mPFC regions to DRN target inhibitory GABAergic neurons [12,16]. While it is not clear whether the mPFC–DRN pathways stimulated in this study targeted serotonergic or GABAergic cells, it appears that the convergent findings obtained through direct DRN stimulation likely did target a combination of these neuronal populations, making the net effect on serotonin release uncertain. Moreover, in the case of mPFC–LHb stimulation, the possibility that this impacted behavior through the serotonergic DRN must be weighed against the alternative or additional possibility that decreased mobility obtained through LHb's well-known inhibitory projections to the dopaminergic midbrain [15].

Notwithstanding these residual ambiguities, the Warden *et al.* [3] study does provide compelling new support for an important role of prefrontal-neuromodulatory pathways in regulating behavioral responses to environmental stressors. Perhaps more strikingly, the work provides clear-cut evidence for dramatic effects of DRN and LHb activity on behavior, at surprisingly brief time-scales. A key in demonstrating these effects, and tracking them to specific neural pathways, was the application of optogenetic control, a relatively new technique for selectively activating or 'silencing' a particular population of neurons, such as those projecting from mPFC to DRN. Combined with new developments for stimulating and recording from animals engaged in vigorous underwater movement, the authors were able to show rapid onsets and offsets of behavioral change relative to periods of stimulation. This paper [3] thus provides a compelling demonstration of how these tools can be used to pursue long-standing questions with greater control and precision. Given the clear, but still poorly understood links connecting both mPFC and serotonergic function with clinical disorders like major depression [17–19], these advances also raise hope for new progress on the translational front.

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