a hint that some combination of dopaminergic neurons may supply the mechanism by which thirst gates memory expression. This idea is reminiscent of the known role for MB-MP1 dopamine neurons in gating sugar reward memory expression in satiated flies[14].

In *Drosophila*, water seeking only occurs in water-deprived animals. Drinking is rewarding to flies, but only when they are thirsty. Memories of previous water rewards are only expressed when the internal state is one of thirst. Moreover, when these new findings with water memory are viewed in the context of the larger literature on olfactory memory in flies, the theme that emerges is that olfactory memory relies on the same MB structure irrespective of the modality or value of the reinforcement. The specificity of the experience and the effect of internal state arise from the distinct neuromodulatory input neurons and their restricted zones of contact with different subsets of MB intrinsic neurons. The so-called lovers of dew are underestimated by their name. Instead of thoughtlessly yearning for the morning dew, fruit flies exhibit plastic responses to water and to their levels of thirst.

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Bilingual neurons release glutamate and GABA

Naoshige Uchida

A study finds evidence supporting co-release of glutamate and GABA, excitatory and inhibitory fast neurotransmitters, from a single axon terminal in neurons of the ventral tegmental area that project to the lateral habenula.

Dale's principle, promoted by Sir John Eccles[1], postulates that the same chemical transmitter is released from all of the synaptic terminals of a neuron. In other words, each neuron produces a single neurotransmitter and the identity of this neurotransmitter never changes. Those neurons that release glutamate excite their postsynaptic partners, those that release GABA inhibit them and those that release dopamine 'modulate' them, for example, by modifying the efficacy of synaptic plasticity. Increasing evidence suggests, however, that Dale's principle does not always hold true: some neurons release multiple neurotransmitters[2,3] and some change their neurotransmitter identity[4]. In a study published in this issue of *Nature Neuroscience*, Root *et al.*[5] add yet another astonishing case: a large fraction of rodent ventral tegmental area (VTA) neurons that project to lateral habenula (LHb) co-release glutamate and GABA, two main excitatory and inhibitory fast neurotransmitters, from single axon terminals.

The VTA is one of the main sources of dopamine in the brain, but it also contains neurons that release other neurotransmitters. The authors first examined the neurotransmitter identity of VTA neurons that project to the LHb (**Fig. 1a**) by retrogradely labeling neurons from LHb and staining for neurotransmitter markers at their cell bodies in the VTA. The authors found that most (~80%) of the LHb-innervating VTA neurons coexpressed markers for glutamate and GABA signaling: vesicular glutamate transporter 2 (VGluT2, an enzyme that loads glutamate into synaptic vesicles)

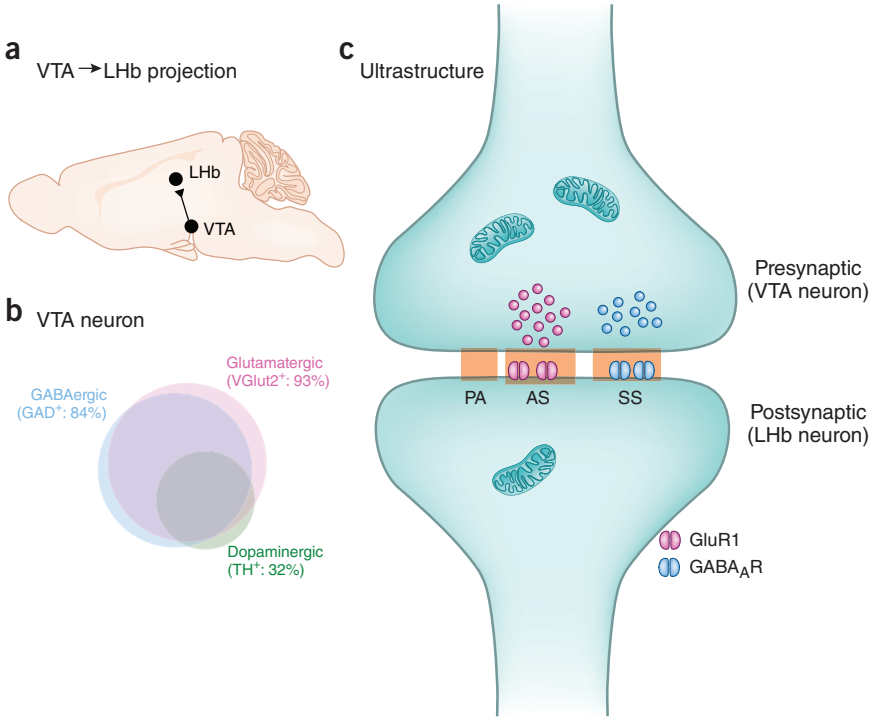

Figure 1 LHb-innervating VTA neurons co-release glutamate and GABA from single axon terminals. (**a**) VTA-to-LHb projection. (**b**) A Venn diagram illustrating the proportions of neurons that express markers for glutamatergic (VGlut2$^+$), GABAergic (GAD$^+$) and dopaminergic (TH$^+$) neurons. Areas shown are approximate. (**c**) Schematic drawing of the ultrastructure of a synapse between a VTA neuron axon and a postsynaptic LHb neuron. AS, asymmetric (putative excitatory) synapse; PA, punctum adherentium; SS, symmetric (putative inhibitory) synapse.

Naoshige Uchida is in the Center for Brain Science, Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA.
e-mail: uchida@mcb.harvard.edu

for glutamate, and glutamate decarboxylase (GAD65/67, two subunits of an enzyme for GABA synthesis) and vesicular GABA transporter (VGaT, an enzyme that loads GABA into synaptic vesicles) for GABA (**Fig. 1b**). Although some of the LHb-projecting neurons expressed a marker for dopamine neurons (tyrosine hydroxylase, TH) and some of these neurons coexpressed markers for GABA[6] and/or glutamate, the TH-positive population was only ~30% of the total. A previous study[6] also showed that TH-positive neurons do not release detectable levels of dopamine in LHb, suggesting that dopamine has little involvement in VTA to LHb projections.

These results indicate that a large fraction of LHb-projecting neurons have the ability to package glutamate and GABA into synaptic vesicles, but it is unclear whether they are released from the same axon terminals, from the same transmitter release zones or from the same vesicles. Using immunoelectron microscopy, the authors showed that a majority of axon terminals of LHb-projecting VTA neurons were positive for both VGluT2 and VGaT, indicating that both glutamate and GABA are released from single axon terminals. Morphological analyses at an electron microscopic level showed that these axon terminals formed multiple synapses on the dendritic spines and shafts of LHb neurons. Surprisingly, the authors found that single axon terminals established both asymmetric (putative excitatory) and symmetric (putative inhibitory) synapses (**Fig. 1c**). The postsynaptic sides of asymmetric and symmetric synapses contained receptors for glutamate and GABA (glutamate 1 AMPA-type receptor and $GABA_A$ receptor), respectively, further supporting the excitatory and inhibitory natures of these synapses (transmitter release zones). These results suggest that LHb-innervating VTA neurons form functional glutamatergic and GABAergic synapses at distinct microdomains in a single axon terminal.

Next the authors examined these connections electrophysiologically. The light-gated cation channel channelrhodopsin-2 (ChR2) was expressed in VTA neurons that express glutamatergic genes (*VGluT2* (also known as *Slc17a6*) in mice or *Camk2a* in rats). In slice preparations, the authors optogenetically stimulated the axons of VGluT2-positive VTA neurons while measuring the postsynaptic responses in LHb neurons using whole-cell patch-clamp recording. Consistent with the above morphological analyses, optogenetic stimulation of these fibers evoked both excitatory and inhibitory synaptic responses. These responses were diminished by the sodium channel blocker tetrodotoxin, but recovered after boosting depolarizations with the voltage-dependent K^+ channel blocker 4-aminopyridine. These results show that neither the excitatory nor the inhibitory responses required action potentials, and they are therefore likely to reflect monosynaptic connections.

Next, the authors examined how activation of these projections modulates firing of LHb neurons in an intact animal. The authors recorded spiking activity of LHb neurons using extracellular recording while optogenetically activating the axons of either VGluT2- or VGaT-positive VTA neurons in anesthetized mice (the authors also tested using CaMKIIα-positive neurons in anesthetized rats). Single 10-ms stimulations of these axons caused multimodal responses consisting of periods of increased and decreased spiking: fast excitation followed by inhibition, fast excitation alone, fast inhibition alone or fast inhibition followed by excitation. Initial responses were more often (~70%) inhibitory.

Together, these results provide compelling evidence of glutamate and GABA co-release. Remarkably, this co-release appears to occur from single axon terminals containing separate, yet adjacent, neurotransmitter release zones for either glutamate or GABA. Glutamate-GABA co-release is rare, but it has been reported previously[7,8]. So-called mossy fiber synapses in the hippocampus (the synapse between granule cells in the dentate gyrus and pyramidal neurons in CA3) are glutamatergic, but appear to transiently acquire the ability to release GABA after epileptic activity (perhaps serving an anti-epileptic function) or during development[7]. Notably, Root *et al.*[5] used adult animals, both rats and mice, under conditions of normal activity, and still found co-release. Furthermore, it is noteworthy that the neural population that releases both glutamate and GABA is the majority, rather than the minority, of LHb-projecting VTA neurons. Finally, the authors showed that these synapses are common in the LHb, as well as that co-released glutamate and GABA may occur in inputs from areas other than the VTA.

What is the role of glutamate-GABA co-release in computation and brain function? Co-release from the same axon terminal means that excitatory and inhibitory inputs convey primarily the same information to the postsynaptic neuron. At first glance, this may seem odd, but there might be some advantage that could be garnered by glutamate-GABA co-release. Although future studies should address this question, here I speculate in light of the presumed functions of LHb.

First, the ability to independently modulate the strength of glutamatergic and GABAergic synapses may provide flexibility in regulating the balance between the two and, ultimately, the excitability of postsynaptic neurons (as seen in mossy fiber synapses). Second, balanced inputs may set the 'gain' of postsynaptic neurons. Computational studies[9] have shown that a balanced increase or decrease of excitatory and inhibitory inputs can alter the gain of a neuron's responses to the input coming from other sources without altering the neuron's baseline firing rate. Having both excitation and inhibition originate from the same source might make it easier to maintain balance between the two, allowing the baseline firing rates of postsynaptic neurons to be tightly regulated. Third, if excitation and inhibition are time-lagged in certain ways, this can produce specific filtering of inputs. For instance, excitation followed by inhibition can be equivalent to taking the derivative of inputs, emphasizing transient inputs while de-emphasizing sustained inputs. Inhibition followed by excitation can be equivalent to taking the derivative and flipping the sign.

The LHb has been implicated in depression[10] and the computation of reward prediction error signals[11]. The possibilities listed above are relevant for these functions. First, the excitability of LHb neurons has been linked to depression[10]. Co-release of glutamate and GABA may provide powerful control over LHb neuron excitability through the plasticity of glutamate and/or GABA synapses or by modulating the gain of driving inputs. Second, LHb neurons are excited by unpredicted aversive events and inhibited by unpredicted reward[11]. These responses diminish when the outcome is expected. Notably, a previous study showed that VTA neurons that express a GABAergic marker (VGaT) exhibit sustained activation during the delay between a reward-predictive cue and reward, suggesting that these neurons signal reward expectation[12]. Although it is unclear whether the neurons recorded in that study[12] projected to LHb or coexpressed VGluT2, these data raise the possibility that LHb-projecting VTA neurons regulate the gain of LHb neurons' responses to outcomes. Furthermore, certain computational models, such as temporal difference models[13], posit that taking the derivative of reward expectation signals (that is, taking the derivative of the value function or calculating the difference in value functions at consecutive time points) is a key step in computing reward prediction errors[14]. Co-release of glutamate and GABA may provide a mechanism to obtain the derivative function to facilitate prediction error calculations.

In summary, Root *et al.*[5] provide strong evidence for glutamate-GABA co-release from single axon terminals. This projection represents a

major violation of Dale's principle. This exciting finding raises important questions about what computational advantages, if any, this co-release offers for the function of the LHb.

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Cortical adaptation and tactile perception

Hongdian Yang & Daniel H O'Connor

Cortical neurons reduce spiking responses to repetitive sensory stimulation, but the perceptual impact of this adaptation has been difficult to assess. Work now shows that it has profound consequences for tactile perception.

Are you sitting down? If so, you probably weren't aware—until now—of how your chair feels on your body. During prolonged or repetitive stimulation, the responses of many sensory neurons decrease over time. Previous studies in the rodent whisker-barrel system have shown how sensory adaptation can affect the detection or discrimination of sensory stimuli[1,2]. However, this work has been limited by the coupling between adaptation and sensory stimulation. In this issue of *Nature Neuroscience*, Musall *et al.* study perceptual behavior in the absence of adaptation using optogenetic stimulation, directly tackling the question of how adaptation of rodent primary somatosensory (S1, specifically barrel) cortex neurons affects perceptual decisions[3]. They discovered that after adaptation is abolished, some perceptual abilities are dramatically improved, including the ability to discriminate between frequencies of tactile stimulation. Other perceptual abilities suffer, in particular the ability to detect oddball stimuli within ongoing patterns of tactile stimulation. Cortical adaptation thus likely influences perception in multiple and profound ways.

Understanding the perceptual consequences of adaptation requires behavioral reports in animals performing sensory tasks. To this end, Musall *et al.*[3] trained rats in tasks involving either simple detection of tactile stimuli or discrimination of different frequencies of whisker stimulation. Adaptation is a prominent feature of neural responses in S1 to the stimuli used in this task[3,4].

Hongdian Yang and Daniel H. O'Connor are in the Solomon H. Snyder Department of Neuroscience & Brain Science Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
e-mail: dan.oconnor@jhmi.edu

Musall *et al.*[3] found that optogenetic stimulation in S1 using channelrhodopsin-2 (ChR2) can substitute for tactile stimulation. This further extends a powerful tradition of electrical microstimulation[5] of S1 into the realm of cell type–specific optogenetic stimulation during tactile behavior[6,7]. Calibrated optogenetic stimulation gave the authors a 'volume control' with which to dial up the degree of adaptation during repetitive pulse trains that otherwise largely mimicked whisker-evoked responses. The authors could then compare the rats' performance in the presence of adaptation—the normal case—using conventional whisker deflection with their performance in the absence of adaptation using direct optogenetic stimulation of S1 neurons.

To begin, the authors quantified the performance of each rat in detecting a whisker deflection as a function of whisker stimulation velocity, finding the stimulus strength for each rat that yielded a perceptually difficult threshold stimulus (the velocity that was halfway to maximal detectability). The authors then asked how the ability of the animal to detect this threshold stimulus depended on the number of stimulus repetitions within a trial. That is, one stimulus pulse is difficult to detect, but how much easier are two pulses, three pulses and so on? Repetitions were given at 40 Hz, a frequency subject to strong adaptation[4]. The authors found that pulses beyond the first added little to the probability that the rat would detect the stimulus train; even four pulses were only slightly more detectable than a single pulse. This result contrasts sharply with what one would expect if each pulse contributed equally to detection.

Adaptation reduces overall spike count because pulses occurring later in a train evoke fewer spikes. If rats perform the detection task by monitoring overall spike count, the above behavioral result makes sense: pulses after the first in a 40-Hz train yield relatively few additional spikes. Indeed, the authors could predict the relationship between detection performance and the number of pulses using a model that accounted for the reduced spike count caused by adaptation. This was a simple detection task. But how would adaptation affect perceptual judgments of a very different sort, in which stimulation frequency is critical?

To address this, the authors trained rats to discriminate between different frequencies of whisker stimulation. A stimulus train comprising deflections at a target frequency was delivered to a whisker on one side of the face while a distractor stimulus of lower frequency was delivered simultaneously to a whisker on the other side. The lowest-frequency distractor stimulus comprised a single pulse within the stimulation period. As expected, the closer the stimulation and distractor frequencies, the worse the rats' performance was. Remarkably, even a single distractor pulse could dramatically damage performance. This behavioral result could be explained by a model of the decision process based on spike count: because of adaptation, the largest component of the neural response to a train of any frequency is the first-pulse response. Because this dominant first-pulse response is the same for all frequencies, even a single pulse will substantially interfere with discrimination performance. In contrast, if animals were explicitly monitoring the interval between stimulus responses, the large effect of even a single distractor would be harder to explain. This experiment provided further evidence that adaptation critically affects touch perception, even in the context of frequency discrimination.