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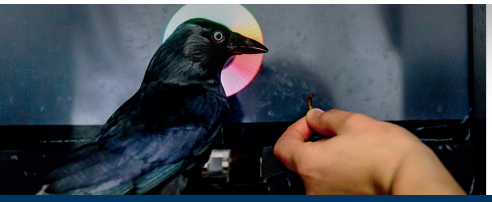
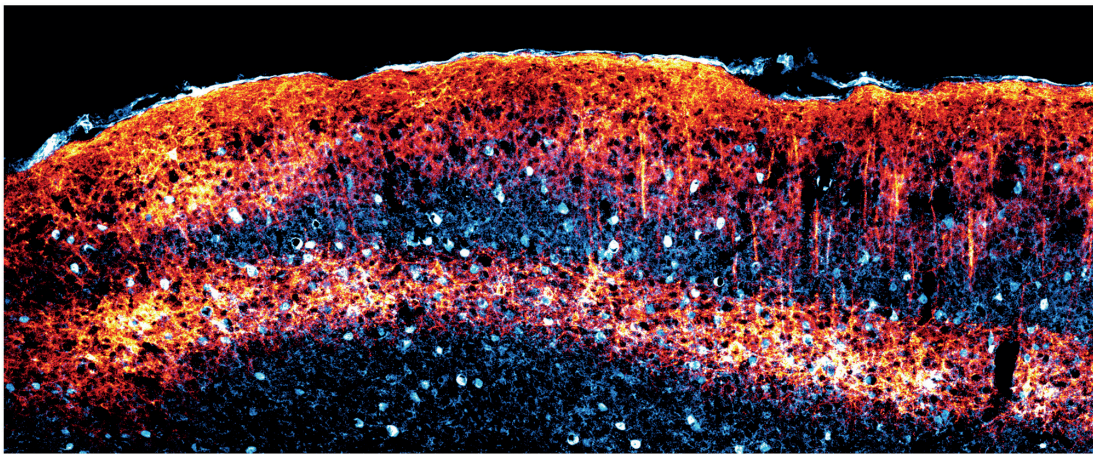
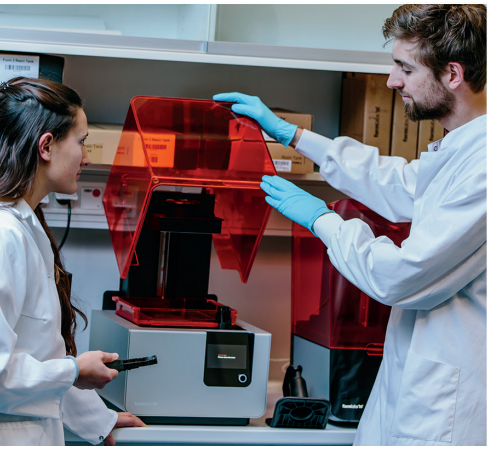
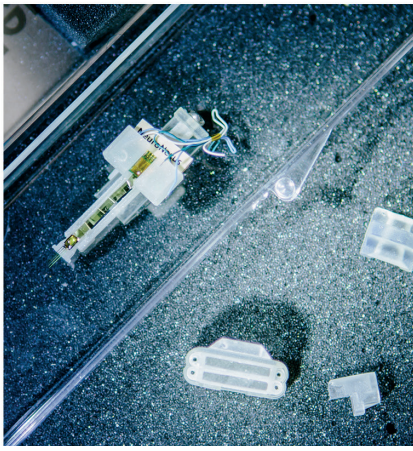


INTEGRATION AND REPRESENTATION OF SENSORY PROCESSES







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SEROTONIN: MORE THAN A HAPPY HORMONE

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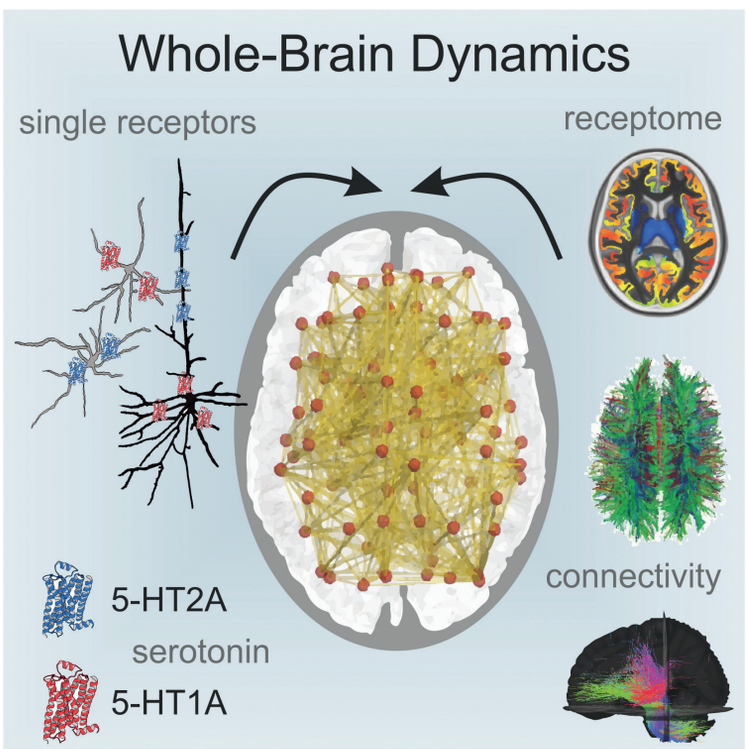
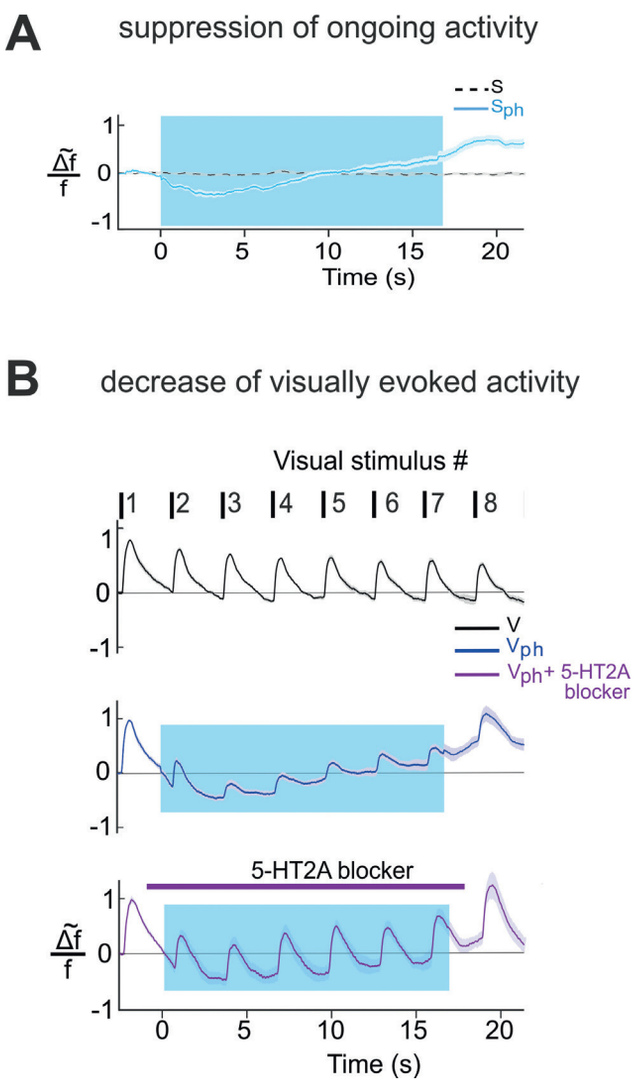


◂ **Figure 1:** How neuromodulators influence entire brain dynamics remains elusive. We propose a new 'receptome' approach that combines the brain's anatomical and functional constraints with Michaelis–Menten kinetics at individual receptor sites (Kringelbach et al., 2020). Exemplified by the serotonergic (5-HT) system, we show how optogenetic tools can be used for evaluation of our hypotheses in animal experiments. Our approaches may trigger new ways for the diagnosis and treatment of psychiatric disorders associated with malfunction of neurotransmitter systems. Modified from Jancke et al., 2021

Serotonin (5-HT) is best known as a *happy hormone* and a mood regulator. Eating delicious food, receiving a job promotion, or doing sports can all increase your brain 5-HT levels (Young, 2007). On the other hand, irregularities in 5-HT signalling can lead to neuropsychiatric disorders such as anxiety, depression and addiction (Lucki, 1998; Figure 1). However, 5-HT is present not only in brain regions involved in mood and reward, but also in areas of the brain that process incoming information from our senses (Dugue et al., 2014; Lottem et al., 2016). So, could it be that 5-HT also directly regulates perception?

Optogenetics – Using light to control the brain's computation

The Dorsal Raphé Nucleus (DRN), located in the brainstem, is the primary source of 5-HT for the brain (Ishimura et al., 1988). The DRN sends its axons in a widely distributed manner across various brain areas (Waterhouse et al., 1986). Optogenetics, a technique that allows the expression of light-activated proteins in targeted classes of neurons, can be used to manipulate (switch on or off) individual types of neurons in the brain by photostimulation (Boyden et al., 2005; Li et al., 2005). Thus, using this state-of-the-art method, serotonergic neurons in the DRN can be selectively activated, and the role of 5-HT signalling can be investigated in several brain areas receiving input from the DRN. For instance, a recent study showed that optogenetic DRN stimulation, which results in elevated brain levels of 5-HT, leads to a decrease in mechanosensory responsivity in mice, and an increase of the detection threshold for tactile stimuli (Dugue et al., 2014). Another study in the mouse olfactory cortex showed that release of 5-HT from DRN neurons inhibits spontaneous cortical activity (i.e., ongoing fluctuations in activity that reflect internal brain broadcasts), while leaving the actual olfactory stimulus-driven activity unchanged (Lottem et al., 2016). These findings provide strong evidence that 5-HT signalling can differentiate between different streams of sensory information and can selectively alter their relative weight, thereby modulating their ▸

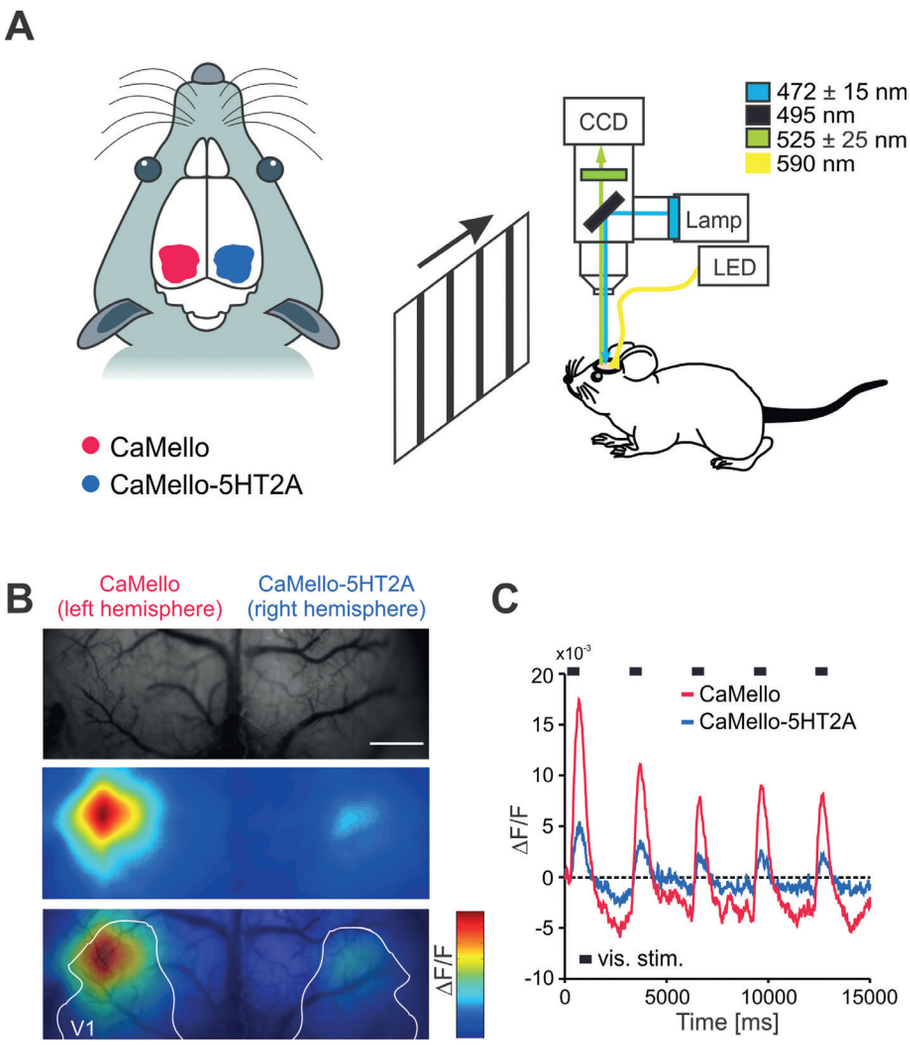


◂ **Figure 2:** V1 activity modulation by serotonergic input (A) Calcium imaging recordings in V1 show that activation of serotonergic neurons suppresses the spontaneous ongoing activity in V1. The black stippled line indicates spontaneous activity without photostimulation (S). The blue solid line shows spontaneous activity with simultaneous DRN photostimulation (Sph). The blue rectangle indicates the time span of photostimulation. (B) Top: visually evoked activity (V) in response to 8 visual stimuli, indicated by the vertical bars on top. Middle: Same as above, with photostimulation of the 5-HT neurons (Vph). Bottom: visually evoked activity during photostimulation of 5-HT neurons while antagonising the 5-HT2A receptor (Vph+5-HT2A blocker). Please note how the visual responses gain is rescued by the blocker application, while the suppression of the baseline is not affected. Modified from Azimi et al., 2020

impact on other ongoing communication processes that are permanently at work in our brains.

Precise triggering of 5-HT release by light provides insight into visual cortical processes

Intrigued by these results, we aimed to find out what role 5-HT plays in visual information processing. While optogenetically stimulating 5-HT neurons in the DRN, we recorded neuronal activity in the mouse primary visual cortex (V1) using calcium imaging and extracellular recordings (Azimi et al., 2020). Calcium imaging allows us to monitor neuronal activity based on the following principles: when a neuron becomes active (referred to as 'neuronal firing'), calcium channels located in the neuronal membrane open. Calcium ions then enter the neuron causing an elevation in intracellular calcium concentration. If we attach a calcium sensor to the neurons, that contains a fluorescent protein, then every time the neuron fires we will see a flash of light that can be and recorded optically with a highly sensitive charge-coupled device (CCD) camera. Thus, fluctuations in neuronal activity can be translated into subtle changes in the fluorescence of this calcium-binding protein (in our case: the RCaMP calcium sensor). By these means, the CCD camera can capture changes in activity of thousands of neurons across several millimetres of cortex at the population level. Our results show suppression of spontaneous cortical population activity in V1 upon 5-HT release (Figure 2A). This implies, that increased 5-HT levels dampen the relative weight of internally ongoing broadcasts. Next, we presented visual stimuli (in the form of horizontal or vertical gratings) to our subjects (adult mice) and recorded activity in V1 that was triggered by these visual inputs, in the presence, or absence, of optogenetic stimulation of the DRN. We found that the gain (magnitude) of the visually evoked responses was also decreased by 5-HT release (Figure 2B). Altogether, this suggests that 5-HT reduces both the external sensory drive and internal ongoing activity, overall leading to a balanced reduction of both components of brain activity. To investigate more closely the mechanisms by which 5-HT affects network activity in V1, we pharmacologically manipulated the activity of the most abundant 5-HT receptor in the mouse visual cortex, the so-called 5-HT2A receptor. Interestingly, the experiments revealed that activation of the 5-HT2A receptor specifically mediates the decrease in the visual response gain, while it has no effect on the ongoing activity. These findings prove once again the versatility of 5-HT acting as a neuromodulator of the cortical state, orchestrating a fine-tuned scaling between external sensory drive and internal broadcasts. Such interplay could be further modulated by different expression patterns of the various



◂ **Figure 3:** Simultaneous photostimulation and calcium imaging of visually evoked responses in V1 in vivo. (A) Scheme of the experimental setup. The optogenetic probes were activated with blue light (472 +/- 15 nm) and deactivated with yellow light (590 nm) between recording sessions. (B) Top: view through the skull of the imaged area, where the dark patterns represent blood vessels. Middle: GCaMP signal in response to visual stimulation. Bottom: overlay of the two images above. Scale bar = 1 mm. (C) Traces represent the time course of spatial averages over the V1 for CaMello (control probe) and CaMello-5HT2A (test probe), as relative change in fluorescence (ΔF/F). Black bars on top visualise stimulus timing. Modified from Eickelbeck et al., 2019

serotonergic receptor types, depending on cellular compartment, cell type and brain region.

Going one step further, we used two optogenetic tools that were developed 'in-house' to test the modulatory effect of activating exclusively the 5-HT2A receptor (Eickelbeck et al., 2019). The used control optogenetic probe (CaMello) consisted of a light-activated protein found in the retina and a GCaMP calcium sensor that allowed us to monitor neuronal firing activity using our CCD camera. However, the test probe that we used (CaMello-5HT2A) included also the C-terminal region of the 5-HT2A receptor, which targets CaMello to the cellular domains, where endogenous 5-HT2A receptors reside. Upon illumination of the mouse cortex through the skull, we could activate both the control and the test optogenetic tool, while simultaneously recording calcium signals reported by GCaMP (Figure 3). Our results show that the additional expression of the 5-HT2A C-terminal region decreases the visual response gain, in comparison to the control probe. These findings support our hypothesis, that the endogenous 5-HT2A receptor largely modulates the weight of visually evoked activity and thus, controls the impact of incoming visual information (Azimi et al., 2018; Michaiel et al., 2019; Azimi et al., 2020).

What can we learn from these studies? Firstly, in addition to obtaining valuable mechanistic insights, we gained knowledge that a single type of 5-HT receptor, here the 5-HT2A receptor, can be responsible for a massive reduction in activity driven by external visual stimuli. This means that in turn, the relative weight of internally-driven ongoing broadcasts is increased. Hence, in extreme cases hallucinations may be caused, which are assumed to reflect an overly high impact of internal brain communication processes compared to incoming information from the external senses (Figure 4). Intriguingly, LSD (Lysergic acid diethylamide) a powerful psychoactive drug that produces visual hallucinations, mainly targets the 5-HT2A receptor. Considered from the perspective of our main research question mentioned above, we can conclude that 5-HT has indeed an important function in sensory processing and perception.

Secondly, we learned that 5-HT produces changes in brain state, that is, changes in neuronal dynamics through which large networks of neurons located across the brain communicate with one another. Consequently, changes in brain state will critically depend on individual distributions of 5-HT receptor densities. Because such distributions depend to a large extent on genetically predetermined expression patterns, our findings may help to better understand the structure and fundaments of psychiatric disorders associated with malfunction of the 5-HT system (see below clinical relevance of our study).

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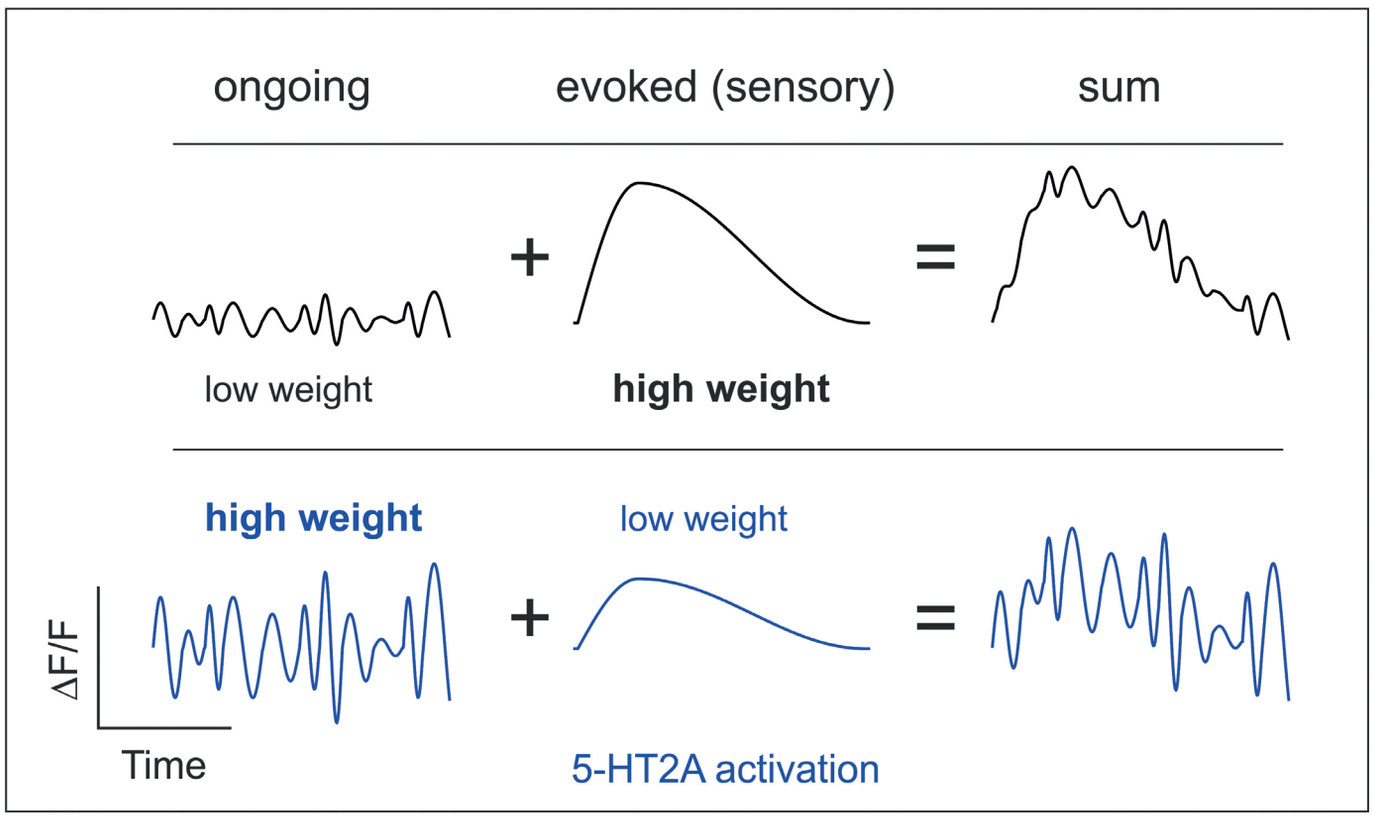

▲ **Figure 4:** Integration of internal ongoing activity and sensory-evoked input with different weights. Top: Summation of downscaled ongoing activity (left) and sensory-evoked input (middle) leads to emphasis on the sensory-evoked activity (right).
Bottom: Summation of upscaled ongoing activity (left) and sensory drive with reduced gain (middle) leads to emphasis of internal brain broadcasts (right), increasing the chance of hallucinations. Modified from Jancke et al., 2021

Future research

To further disentangle 5-HT signalling, we aim not only to specifically activate single serotonergic receptor types across neuronal networks, but also to activate them in selected cell classes. Similarly, to the above described experiments (Eickelbeck et al., 2019), by coupling a 5-HT receptor to a light-activated protein, we can obtain modified serotonergic receptors that can be switched on and off by light. Moreover, we can target the expression of these hybrid receptors to certain classes of neurons, which allows us to manipulate single receptors in a specific cell type. These experiments will shed light onto the neuromodulatory mechanisms of serotonergic receptors in general, and in particular, on how their modulatory effects depend on their expression patterns and distribution across neuronal circuitries.

Clinical importance of the study

An unbalanced expression or activation of serotonergic receptors has been linked to psychiatric disorders like anxiety, depression, schizophrenia and post-traumatic stress disorder (PTSD). 5-HT affects the integration of actual sensory input (bottom-up) with ongoing internal processes (top-down). The 5-HT2A receptor is a target of psychedelic drugs and its activation can emphasise more the internal brain activity in the detriment of the external information in V1 (Michaiel et al., 2019). Such a mechanism can explain the occurrence of hallucinations in disease or drug consumption. The experimental work is backed-up by models of single receptor signalling at the whole brain level (Kringelbach et al., 2020). Ultimately, understanding the complex nature of neuromodulation by serotonin signalling can contribute to designing therapeutic strategies targeting single receptor types with high specificity (Jancke et al., 2021).

Glossary

C-terminal region: The end of an amino-acid chain (protein or peptide), terminated by a free carboxyl group (-COOH). This part of the chain is responsible for directing proteins to specific target regions within a cell.

Calcium imaging: The method measures optically the calcium levels in a cell or tissue using fluorescent indicators. As calcium levels vary with neuronal firing, calcium imaging also reports neuronal activity.

Dorsal Raphé Nucleus (DNR): A brainstem nucleus which is the major location the brain of serotonergic neurons.

Optogenetics: A technique used to control the activity of neurons with light. This is achieved by expression of light-sensitive proteins in selected target cells. The light-sensitive proteins interact with the neurons' natural mechanisms involved in controlling electrical activity. As a result, neuronal activity can be experimentally manipulated by application of light, i.e., by photostimulation.

Primary visual cortex (V1): An area of the brain that represents the main "entrance" of visual information from subcortical structures to the cerebral neocortex.

Serotonin (5-HT): A monoamine neurotransmitter, modulating mood, reward, cognition, learning and memory. The serotonergic system is target for antidepressants and psychedelic drugs.

Visual response gain: Visually evoked response change from baseline. For example, it can be quantified as number of spikes (measured with electrophysiology) or relative fluorescence change (by performing an imaging technique).

Weight: A numerical coefficient assigned to an item to express its relative importance.

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