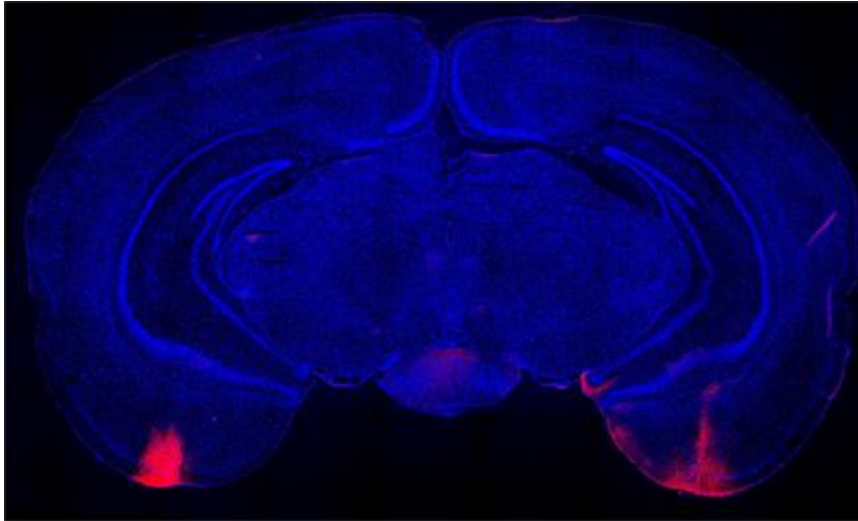


AmPir as a Cape Fear for mice

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Neurons from the amygdalo-piriform transition area (AmPir; red) are involved in stress hormone release by predator odors. Neurons in the AmPir were infected with an adeno-associated virus (AAV) that expresses mCherry fluorescent protein.

Image provided by Dr. Kunio Kondoh.

A wide range of animals exhibit innate fear responses to predator cues. These responses are both behavioral and physiological in nature and include increases in levels of stress hormones in the blood. While neurons expressing corticotropin-releasing hormone (CRH) located in the paraventricular nucleus of the hypothalamus (PVN) are known to be involved, the identity of the brain circuits that relay predator signals from the nose to CRH neurons remains unknown. Most sensory signals travel from the nose to ultimately be interpreted by the olfactory cortex (OC). Like other sensory brain regions, the OC is comprised of distinct anatomical areas, whose functions remain unclear. A new Fred Hutch study led by Drs. Kunio Kondoh and Zhonghua Lu, post-doctoral fellows in Dr. Linda Buck's Laboratory (Basic Sciences Division), combined viral tracing with chemogenetic manipulation of neuronal activity to show that a small region of the mouse OC, known as the amygdalo-piriform transition area (AmPir), is a central component of the hormonal response to volatile predator odors. This study was recently published in *Nature*.

To test whether a specific OC area(s) control(s) stress responses to predator odors, the investigators developed two viral tracing systems wherein Cre-dependent expression of thymidine kinase permits viral replication of thymidine kinase-deficient Bartha strain pseudorabies virus across either one (PRVB316) or multiple (PRVB177) upstream neurons through synapses. To map what OC areas transmitted signals to CRH neurons, the researchers injected the viral tracers, which can only travel retrogradely across synapses, into the PVN of mice that only express Cre in CRH

neurons. Upstream neurons that communicate with the CRH neurons should become pseudorabies virus positive (PRV+). While virus-infected neurons (PRV+) were detected in multiple brain areas outside the PVN and OC areas, only PRV+ neurons located in AmPir exhibited robust neural activity (visualized by *in situ* hybridization for nuclear Arc) in response to predator odors. To test the role of AmPir neurons in the hormone-dependent fear response, the investigators used a chemogenetic strategy to activate AmPir neurons. To this end, they injected adeno-associated viruses that express the receptor hM3Dq, which depolarizes and activates neurons in the presence of a ligand (clozapine-N-oxide; CNO). Remarkably, activation of AmPir led to a 7.6 fold increase in blood levels of adrenocorticotrophic hormone (ACTH), even in the absence of predator odors. The authors turned once again to chemogenetics to address whether AmPir was required for a robust response to predator odors. AmPir-restricted injection of adeno-associated viruses expressing the hM4Di receptor, which silences neural activity upon binding to CNO, resulted in a marked decrease in ACTH plasma levels in response to predator odors from either foxes (2,5-dihydro-2,4,5-trimethylthiazoline) or bobcats (urine). Importantly, silencing AmPir neurons did not affect predator odor-induced immobilization or freezing, a stereotypical behavior caused by predator odors, suggesting that distinct OC areas are responsible for either behavioral or hormonal effects of predator odors. Said Dr. Kondoh " With our new trans-synaptic viral tracing system, we found that AmPir plays an important role in stress hormone release in response to predator odors. We are now interested in how AmPir regulates stress hormone response to predator odors in more detail. Altogether, results from this study showed that while multiple OC areas are competent to transmit signals to CRH neurons, only a small area known as AmPir responds to fox and bobcat odors, suggesting that AmPir is a key component of the hormonal fear response circuit. Because instinctive fear responses to predator odors appear to be an innate rather than learned trait, this study also suggests that the combination of viral tracing, neural activity monitoring and chemogenetic manipulation of neurons will collectively enable other stereotyped circuits to be mapped.

[Kondoh K, Lu Z, Ye X, Olson DP, Lowell BB, Buck LB](#). 2016. A specific area of olfactory cortex involved in stress hormone responses to predator odours. *Nature*. 532(7597):103-106.

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