Neural activity of reward processing and anhedonia as a biomarker for adolescent and early adulthood depression: the role of stress in a translational approach

Marco Cambiaghi, PhD
Alexander Bodini Research Fellow in Developmental and Adolescent Psychiatry
The Italian Academy for Advanced Studies in America, Columbia University
Neural Circuits Lab, Columbia University and the New York State Psychiatric Institute

> "*It may be that there is a time in life when one is tired of everything and feels, perhaps correctly, as if all one does is wrong - do you think this is a feeling one must try to avoid and to banish…?*" [1]

Depression has been described by mankind for several millennia. The term melancholia (which means black bile in Greek) was first used by Hippocrates around 400 B.C. (Akiskal, 2000) to describe most of the major symptoms of depression observed today (Nestler et al., 2002). Since the 1960s, depression has been diagnosed as "major depression" based on symptomatic criteria that can vary from mild to severe (Diagnostic and Statistical Manual, 2000).

Depressed mood

Irritability

Low self esteem

Feelings of hopelessness, worthlessness, and guilt

Decreased ability to concentrate and think

Decreased or increased appetite

Weight loss or weight gain

Insomnia or hypersomnia

Low energy, fatigue, or increased agitation

Decreased interest in pleasurable stimuli (e.g., sex, food, social interactions)

Recurrent thoughts of death and suicide

A diagnosis of major depression is made when a certain number of the above symptoms are reported for longer than a 2 week period of time, and when the symptoms disrupt normal social and occupational functioning

[1] Letter from Vincent van Gogh to Theo van Gogh - Dordrecht, 7-8 February 1877

Depression nowadays

Depression is a debilitating, costly, and widespread disorder that affects children as well as adults. Depression affects approximately one in 15 adults (6.7%) in any given year. And one in six people (16.6%) will experience depression at some time in their life. Depression can strike at any time, but on average, first appears during the late teens to mid-20s with 2.2% of 9–16-year-old children meeting criteria for depression in a given three-month period and with an estimated cumulative prevalence of 9.5% by age 16. Early symptoms of depression in youth are perhaps the strongest predictor of adolescent and adult depression, and subthreshold depression in adolescence confers an estimated risk of 67% for a diagnosis of depression by the time individuals are in their early 30s (Bress et al., 2012 Biol. Psychology).

As reported above, according to the American Psychiatric Association (2000) a core diagnostic criterion of depression is the lack of interest in pleasurable activities, called anhedonia. In addition, anhedonia itself has been considered a risk factor increasing vulnerability to depression and, when associated with depression it prognosticates a worse outcome (Pizzagalli et al., 2008 Journal of Psychiatric Research; Bress et al., 2012 Biol. Psychology).

Studies measuring resting brain activity have reported that depression is characterized by a reduction within the prefrontal (PFC) regions that are assumed to play an important role in approach-related affect and in responding to reward-related cues (Pizzagalli et al., 2008 Journal of Psychiatric Research). For instance, adult patients diagnosed with major depression show decreased medial PFC (mPFC) and striatal activation in response to monetary reward and positively valenced visual stimuli, as well as a decreased amygdala activation in response to happy words using fMRI (Diekhof et al., 2008 Brain Research Reviews).

Children with depression show abnormal patterns of reward responsiveness, as well as more general negative biases in memory (Bishop et al., 2004 Memory) and in their interpretation of events, similar to those seen in adults. Reward responsiveness is often evaluated using guessing tasks, in which the participant makes a selection that can result in a win or a loss, but is not directly tied to performance. In contrast to their non-depressed counterparts, who are more likely to choose the chance for a high-magnitude reward in a guessing task than a low-magnitude reward when the probability of receiving the reward is high, 11-year-old boys with depression do not distinguish between the high- and low-magnitude reward options (Forbes et al., 2007 Biological Psychiatry).

The anatomy of the reward system

In neuroscience, the reward system is a collection of brain structures that are involved in the regulation of behaviors associated with desirable, approachable, or intrinsically positive stimuli.

The brain structures underlying the reward system include the ventral tegmental area, ventral striatum (primarily the nucleus accumbens, but also the olfactory tubercle), substantia nigra (i.e.,

the pars compacta and pars reticulata), dorsal striatum (i.e., caudate nucleus and putamen), prefrontal cortex, anterior cingulate cortex, insular cortex, hippocampus, hypothalamus (particularly, the orexinergic nucleus in the lateral hypothalamus), thalamus (multiple nuclei), subthalamic nucleus, globus pallidus (both external and internal), ventral pallidum, amygdala, and the remainder of the extended amygdala (see Figure 1). Connectivity between these areas forms a complex neural network that mediates different aspects of reward processing (Haber and Knutson, 2010 Neuropsychopharmacology).

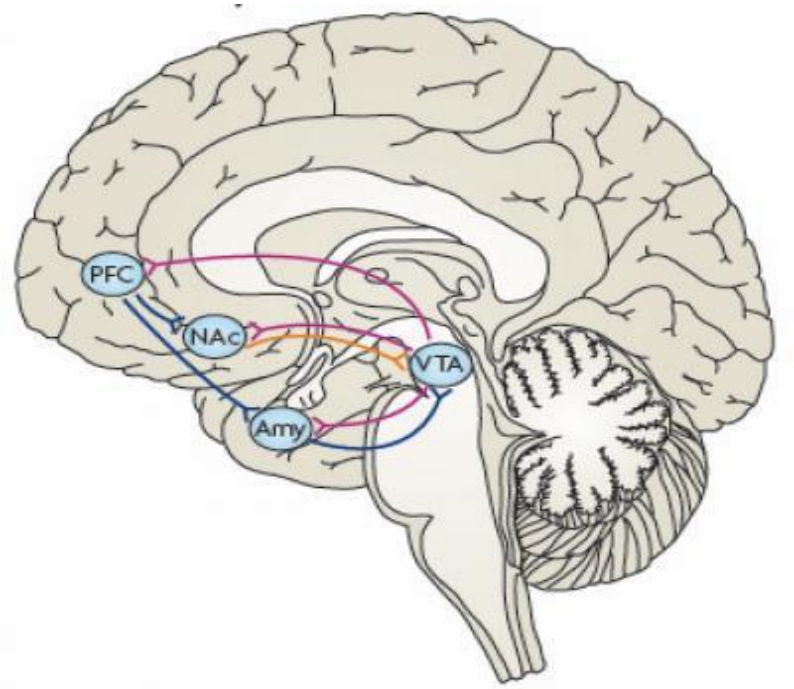


Figure 1. Neural circuitries of reward

A simple schematic of some key regions in the reward circuitries. These regions are highly interconnected and function as a series of integrated parallel circuits that regulate emotional states. Each is heavily innervated by the brain's monoaminergic systems — noradrenaline from the locus coeruleus, dopamine from the ventral tegmental area (VTA) and serotonin from the raphe nuclei — which are thought to modulate the activity of these areas. The amygdala (Amy) is particularly important for conditioned aspects of learning and memory, as is best studied in fear models. The nucleus accumbens (NAc) is a key reward region that regulates an individual's responses to natural rewards and mediates the addicting actions of drugs of abuse. The prefrontal cortex (PFC) — which is composed of multiple regions (for example, the dorsolateral PFC, the medial PFC, the orbitofrontal cortex and the anterior cingulate cortex, among others) with distinct but overlapping functions —is essential to emotion regulation. PFC regions provide top-down control of emotional responses by acting on both the amygdala and the NAc. Some other regions that are important for reward are not shown. Blue lines represent glutamatergic connections; red lines represent dopaminergic connections; the orange line represents a GABA (γ-aminobutyric acid)-ergic connection. (modified from Feder et al., Nat Rev Neurosci. 2009)

Traditionally, rewards are defined as stimuli an organism is willing to work for. On the contrary, punishments are stimuli an organism is trying to avoid. This implies that they are both linked to an operant, such as to an agent's action. According to behaviorist concepts, reward increases the probability that a rewarded behavior is repeated in the future, whereas punishment decreases this probability. Therefore, reward and punishment are closely related to motivation, providing incentives to actively seek or avoid certain stimuli, eliciting appetitive or avoidance behavior, respectively.

Rewards have been categorized into primary and secondary rewards. Primary rewards consist of stimuli which have a direct positive value for an individual receiving the reward. Many of these primary rewards or punishments have a physiological meaning, like food, beverages, sex, and pain. In contrast, secondary rewards have no immediate direct value, but an individual learns that receipt of such rewards usually has positive consequences. Such rewards can be money, tokens, some forms of social acknowledgement, or similar. Valuation of primary rewards depends on hunger, thirst, or other states of the organism, often making it necessary to deprive an individual under observation of the respective reward, in order to make sure that the stimulus is indeed rewarding. In contrast, secondary rewards are less prone to saturation and thus possess a relatively stable value (Lutz and Widmer, 2014 Neuroscience and Neuroeconomics).

So, whereas primary rewards relate directly to survival (e.g., food), secondary rewards are mechanisms of indirect benefit, including monetary gain and social status. In humans, primary and secondary rewards appear to activate the same mesocorticolimbic circuit, including the nucleus accumbens, caudate, putamen, orbitofrontal cortex, and anterior cingulate cortex (Novak&Foti, 2015 Psychophysiology).

Forbes et al. (2006 Journal of Child Psychology and Psychiatry and Allied Disciplines) found that 9–17-year-old boys and girls with depression show less activation in reward-related brain areas (i.e., anterior cingulate cortex, left caudate, and orbitofrontal cortex) than healthy age-matched controls in response to feedback about rewards and losses. These findings suggest that hyposensitivity to reward may be a relatively early-emerging and stable marker of depression (Bress et al., 2012 Biol. Psychology).

The monetary incentive delay task

Among neuroimaging studies, a highly influential experimental paradigm is the monetary incentive delay (MID) task (Knutson et al., 2000 NeuroImage). This task consists of the announcement of an incentive, which is linked with a certain contingency to receipt of this incentive[2]. The MID is designed to explicitly separate neural activity elicited by reward anticipation and outcome, and it has been widely utilized in both basic research and clinical applications. Trials within the MID task proceed across several stages: an incentive cue stimulus

[2] The original paper introducing this new paradigm can be found at this link:
https://www.sciencedirect.com/science/article/pii/S1053811900905937?via%3Dihub

is presented first, one which is explicitly linked with a certain monetary contingency (i.e., potential monetary gain, potential loss, or neutral). Following this cue, a target stimulus is presented that requires a behavioral response. The reward is then delivered depending upon the speed of the individual's response (Figure 2). If contingency exists between an action (i.e., task processing) and a consequence, the learning process rather fits into the scheme of operant conditioning. In this context, appetitive stimuli are called reinforcers, since they strengthen the reinforced behavior. If the action is not reinforced (e.g., because it was not performed to a trainer/teacher's satisfaction), according to learning theory, this leads to extinction.

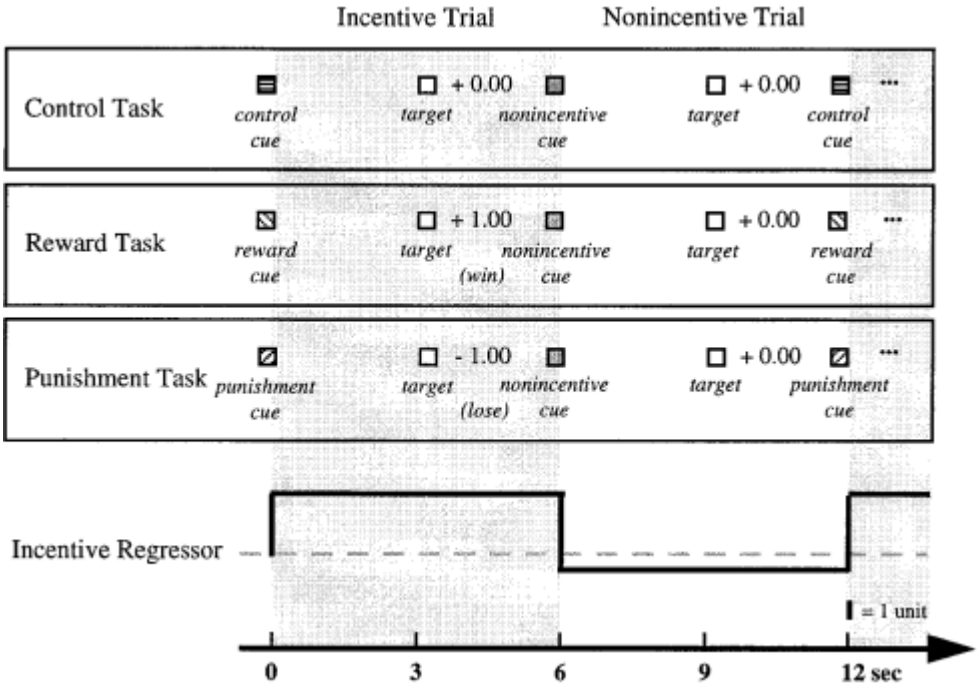


Figure 2. The monetary incentive delay task as presented by Knutson (2000).
Participants are engaged in three consecutive tasks involving either no monetary outcome ("control task"), potential reward ("reward task"), or potential punishment ("punishment task"). Each task consists of 100 6-s trials (10 min total). During each trial, participants see a colored square (cue; 500 ms), wait a variable interval (delay; 4000–4500 ms), and then respond to a white target square which appears for a variable length of time (target; 160–260 ms) with a button press. Immediately after the response, feedback appears (feedback; 500 ms) documenting whether the participant had won or lost money as well as their cumulative sum total at that point. (Knutson et al., 2000)

The MID task has become one of the most widely studied reward paradigms in neuroscience due to its ability to differentiate between stages of reward processing; the initial incentive cue elicits motivated anticipation leading up to the target, and subsequent feedback delivery elicits consummatory processing. The MID paradigm has most often been used with fMRI to isolate patterns of activation during anticipation and outcome. In two studies (Knutson et al., 2000 NeuroImage; Knutson et al., 2001 NeuroReport), both reward and punishment cues elicited a similar pattern of anticipatory activation in striatal areas. Activation specific to reward anticipation was observed in ventral striatal regions, while consummatory activation was

observed in the ventromedial frontal cortex. As expected, no activation was observed for neutral trials in which no reward was possible.

These studies give critical information about the specific brain regions involved in reward processing and provide a foundation for time-locked investigation of anticipatory versus consummatory stages. They also converge with animal research suggesting that anticipatory and consummatory stages of reward may recruit distinct neuroanatomical and neurochemical mechanisms. However, despite its superior spatial resolution, fMRI lacks the necessary temporal resolution to fully delineate the dynamics of reward processing in humans (Novak & Foti, 2015 Psychophysiology). Complementary and more detailed information may be provided by totally non-invasive scalp-recorded measures of brain activity, with millisecond precision, such as electroencephalography (EEG) recordings.

The Adolescent Brain Cognitive Development (ABCD) Study

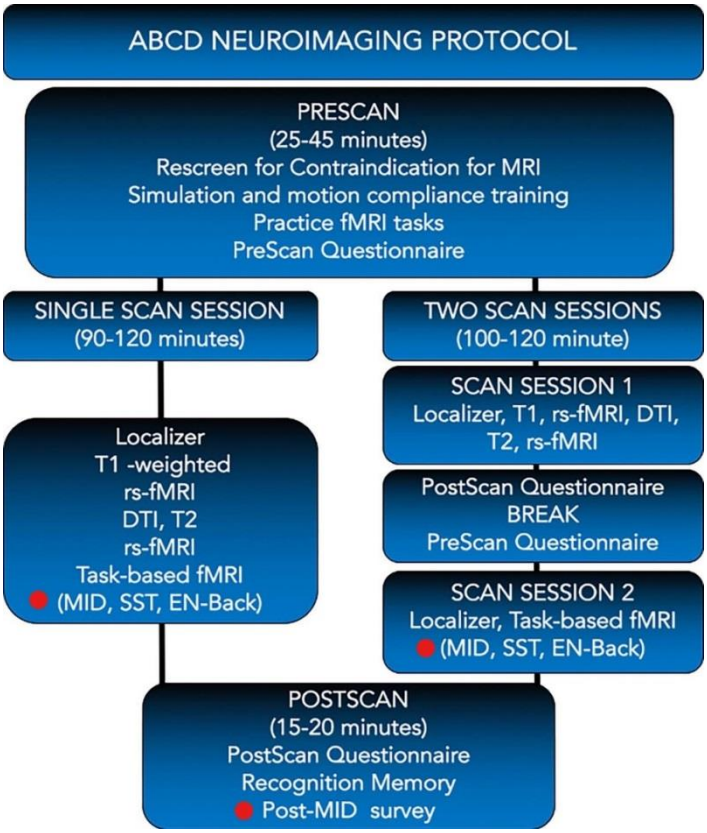
The ABCD Study is a landmark study on brain development and child health that will increase understanding of environmental, social, genetic, and other biological factors that affect brain and cognitive development and can enhance or disrupt a young person's life trajectory. The study, coordinated by the NIH, is being conducted at 21 research sites around the USA and will use advanced neuroimaging to observe brain development in children throughout adolescence, while tracking social, behavioral, physical and environmental factors that may affect brain development and other health outcomes.

The full participant cohort, which was completed on December 3rd 2018, consists of 11,874 youth (age 9-11), including 2,100 young people who are twins or triplets.

Anonymized study data will be made available to the broad research community on a regular basis, allowing scientists to analyze data and ask novel questions that were not even anticipated in the original study planning. Offering these data while the study is in progress means that both ABCD investigators and non-ABCD researchers will have access to the datasets to pursue their own research interests.

There are many health topics that are of interest to researchers, policy makers, educators, parents, and the participants themselves, which scientists can now study using the baseline assessment data. These data can then be tracked over time and used to make predictions as youth develop and are shaped by their life experiences. Scientists can look at brain characteristics associated with impulsive action or early psychopathology (Karcher et al., 2018 JAMA Psychiatry), the impact of health behaviors (e.g., sleep, physical activity) on cognitive and brain development, or traits associated with media use, including screen time exposure. For example, a recent study (Paulus PM. et al., 2019 NeuroImage) by ABCD investigators showed associations between differing amounts and kinds of screen time (e.g., video games vs. social media) and different

structural brain characteristics, psychological traits, and cognitive function. Scientists will be able to follow participants over time to understand how media use will influence a person's development, underscoring the unique opportunity provided by the ABCD study.
Similarly, researchers can look at risk and resilience factors for mental illness and substance use, including genetics, family history, traits such as impulsivity, and exposure to positive and negative environmental events. With longitudinal data, the developmental trajectories of the participants can be tracked to better understand these complex relationships, and eventually to improve prevention or mitigate risks for adverse outcomes[3].



One of the three tasks of the ABCD task-based functional assessment of the brain is the Monetary Incentive Delay task (Figure 3), which will be used to measure the processes of 'anticipation and outcome of reward' (reward processing) and 'anticipation of responding for outcome' (motivation) (Casey et al., 2018 Developmental Cognitive Neuroscience).

Figure 3. ABCD Neuroimaging Protocol. The Monetary Incentive Delay (MID) task is marked with a red spot.

In vivo recording of neural activity

Electroencephalography (EEG) is the measurement of brain electrical fields via electrodes (which act as small antennas) placed on the head. The electrical fields are the result of electrochemical signals passing from one neuron to the next. When billions of these tiny signals are passed simultaneously in spatially extended and geometrically aligned neural populations, the electrical fields sum and become powerful enough to be measured from outside the head. EEG –

[3] More info on the ABCD project can be found at this link: https://abcdstudy.org/

together with magnetoencephalography (MEG) – is the most powerful technique for noninvasively studying the electrophysiological dynamics of the brain, and linking those dynamics to cognition and disease.

In vivo electrophysiological recordings are widely used in neuroscience research, and EEG has become a mainstay of preclinical neuroscience research, including studies of mood behavior, neurological disorders and cognition (Cambiaghi et al., 2015 Seizure; Cambiaghi et al., 2013 Neuropharmacology). Studies utilizing EEG typically involve comparison of measurements obtained from different experimental groups, or from the same experimental group at different times, in which one set of measurements serves as "control" and the others as "test" of the variables of interest. Thus, controls provide mainly a reference measurement for the experimental test. Control rodents represent an undiagnosed population, and cannot be assumed to be "normal" in the sense of being "healthy." Certain physiological EEG patterns seen in humans are also seen in control rodents. However, interpretation of rodent EEG studies relies on documented differences in frequency, morphology, type, location, behavioral state dependence, reactivity, and functional or structural correlates of specific EEG patterns and features between control and test groups.

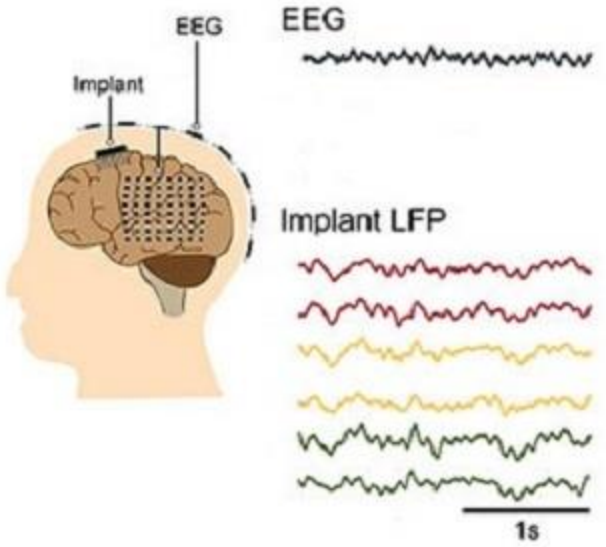


Figure 4. Typical electrophysiological methods. Macroscopic recording via electroencephalography (EEG) and implanted electrodes, with the corresponding representative waveforms recorded in a patient with drug-resistant epilepsy. The measured signal amplitudes are larger implanted electrodes (local field potential or LFP recording) compared to EEG.

EEG records neural activity from outside the brain, and is totally non-invasive. For such a reason, EEG has enormous clinical relevance. In addition to EEG, it is also possible to record deep brain activity, namely the fluctuation of field potentials of different brain areas. This activity reflects the activation of a smaller portion of neurons with respect to EEG large synchronous populations of neurons, and it is called local field potential (LFP) (Figure 4). In animal models, LFP recording is a common technique, together with multiple and single-neuron activity recordings (Cambiaghi et al., 2016 Journal of Neurscience), thanks to the use of small-sized implantable electrodes (Figure 5).

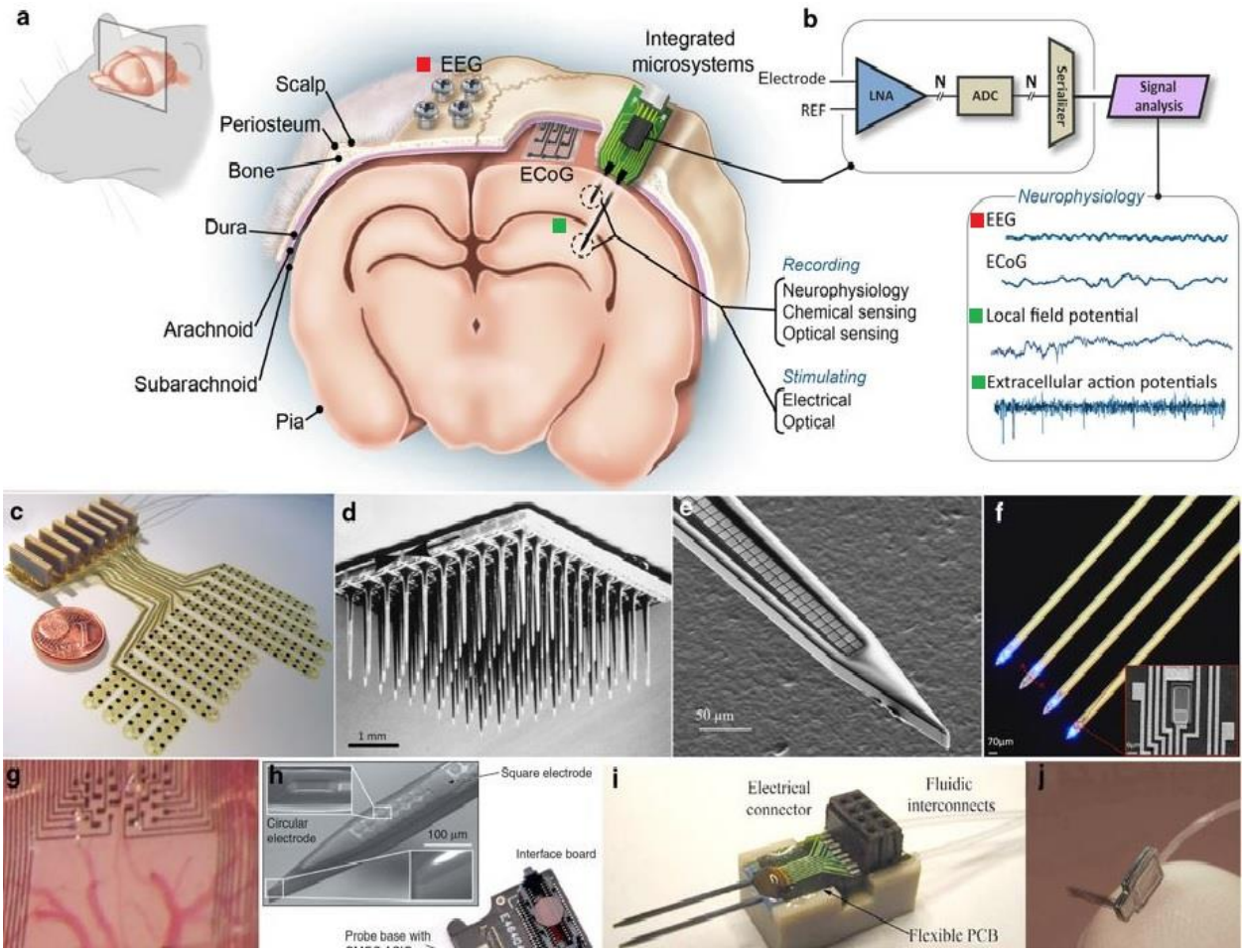


Figure 5. Recording neural activity in the mouse brain

Recording and stimulating technologies vary across scale and degrees of invasiveness. (a) Illustration of the rodent brain and a variety of technologies from electroencephalogram (EEG) to intracortical microelectrodes. (b) High-density systems will increasingly require built-in active electronics to serialize large data streams and reduce the size of the connectors. Sample electrical signals show the amplitudes of various signal sources. The intracortical arrays are often microelectrodes but may also include chemical and optical sensors. (c) Polyimide electrocorticogram (ECoG) for large area mapping. (d) A "Utah array" with 400 µm shank spacing and 100 channels has been used in human studies. (e) Close-packed recording sites with 9×9 µm area and a pitch of 11 µm. (f) MicroLED optoelectrode made from GaN on silicon. (g) Parylene ECoG with greatly improved resolution over EEG and even single-cell capabilities23. (h) CMOS integration on probe shaft and backend. (i) Fluidic probe for drug delivery. (j) Active 3D silicon recording system with flexible parylene interconnect. (from Seymour et al., 2017 Microsystems & Nanoengineering)

The project, *in brief*

Early symptoms of depression in youth are possibly the strongest predictor of adolescent and adult depression In light of the drastic increase in depression from childhood to adolescence, it may be critical to identify early neural markers of reward-related dysfunction that precede the emergence of a full-threshold mood disorder in adolescence and adulthood.

Anhedonia, or decreased enjoyment of reward, is a promising endophenotype of depression which is present in most depressed patients and can be induced in mice. Indeed, mice are susceptible to stress and develop anhedonia (measured as decreased motivation and pursuit of reward) as a consequence (Figure 6).

In rodents, different circuit elements mediate anhedonia, with the ventral tegmental area (VTA) driving anticipation and parts of the nucleus accumbens (NAc) driving consumption. However, it remains unclear how dysfunction in this circuit may lead to anhedonia.

In addition to that, while there have been recent advances in characterizing the neural circuitry that governs depressive behavior in mice, translating these findings to depression biomarkers in humans remains elusive. To address this critical gap, the overarching aim of this project is to use electroencephalogram biomarkers to bridge detailed mouse circuit recordings to adolescent depression.

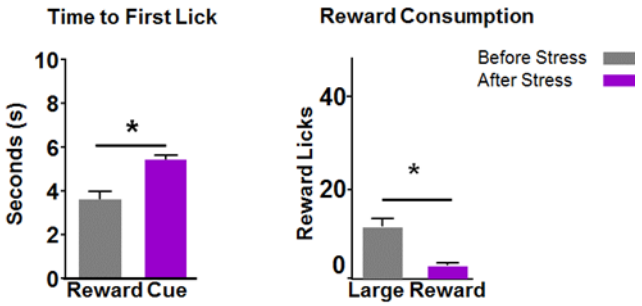


Figure 6. Reward behavior before and after stress

Left. Latency to retrieve reward is shorter before than after acute stress. Right. Mice lick a large reward more before than after acute stress. * $p<0.05$ Wilcoxan sign rank test.

In order to do this, I will train mice a rodent version of the MID, in which monetary gains, losses and neutral conditions are replaced with big, small and medium amount release of water, respectively, in water restricted mice. As in the human MID, cues will signal the contingency of a given trial, with separate tones indicating the potential for gain (big vs medium), loss (small vs medium) or neutral (always medium). To successfully complete a trial, mice will have to nose poke before a transiently appearing light turns off, at which point they can retrieve their reward (Figure 7).

After training, I will implant adolescent mice with 4 skull electrodes to record EEG and intra-cortical electrodes to record local field potentials (LFP) in the VTA, BLA and NAc, and individual neuronal (single unit) firing in the VTA.
The respective intra-cortical recordings appear to have different temporal relationships with the simultaneously recorded surface EEG. These preliminary data illustrate the feasibility of simultaneously recording EEG and intra-cortical activity during reward tasks. Then, I will compare the intra-cortical (namely, LFP) and surface (namely, EEG) elements.
Finally, I will apply this approach to stress susceptible mice. I will subject adolescent mice to a stress protocol and then they will undergo the rodent MID task.

Figure 7. Mouse nose poke.
A light beams register START / END of a nose poke event.

In conclusion, this proposal describes a powerful translational approach for developing depression biomarkers by conducting experiments in adolescent and early adulthood mice, in order to elucidate underlying mechanisms that can be transposed to humans, ensuring a strong clinical relevance. As a final remark, this project plans to conduct parallel experiments in adolescents, in collaboration with Dr. Randy Auerbach (healthy vs depressed adolescents, n=25/group).