Abstract

Stress related mental disorders such as post-traumatic stress disorder (PTSD) or depression have a negative impact on the social interaction of the patients. In the brain, stress response and social interaction are closely linked to the release of the neuromodulator noradrenaline from locus coeruleus into the forebrain. Stress related mood disorders have a destructive effect on the noradrenergic system in the human brain. An up- or down-regulated sensitivity of the locus coeruleus/noradrenergic system to social cues is speculated to cause symptoms like reduced social interest (depression) or aggression (PTSD).

I hypothesize that social dysfunction in mood disorders is at least in part caused by chronic hyperactivation of the locus coeruleus/noradrenergic system during repeated episodes of stress. I further hypothesize that social dysfunction is a consequence of a permanent alteration of locus coeruleus activation after treatments that induce mood disorder-like behavior in rodents.

I will study the alteration in social interaction and its relation to changes in locus coeruleus firing across a repeated defeat treatment. This treatment is known to induce social avoidance in mice. I will therefore submit sexually experienced male mice to this treatment, which under normal conditions would readily engage in courtship and vocalize in response to isolated social odor cues from females. Such context activates the noradrenergic system which regulates arousal in the brain. In a second experiment I will induce chronic hyperactivation in locus coeruleus through pharmaco-genetic manipulation, in order to test whether this alone is sufficient to induce permanent alterations of social behavior and locus coeruleus firing patterns. The behavioral alterations will be measured by quantification of courtship behavior and vocalization. In parallel I will conduct chronic multi-channel recordings in awake mice before and after the treatment.

The pathological impact of stress on the actual activation of neurons in locus coeruleus in social context is poorly investigated, especially in naturally behaving mice. The results of this project are of great importance, in particular because mice are increasingly used to study mood disorders. Further, this project is specifically directed towards the social aspects of stress induced mental conditions. Social stress and social disabilities play major roles in mood disorders.

Proposal: The role of noradrenergic neuromodulation in behavioral models of mood disorders.

The goal of this project is to determine the role of the neuronal firing of noradrenergic neurons in locus coeruleus (LC) in the development of mood disorder-like behavior in mice, with special focus on social interaction. The locus coeruleus/noradrenergic system (LC/NA) regulates arousal and plays a major role in both, stress response and social interaction. **I hypothesize** that repeated stress induces symptoms of mood-disorders through sustained hyperactivation of this LC/NA system. The repeated hyperactivation leads to adaptations in the LC/NA that cause chronically altered response of LC to social stimuli and in consequence to dysregulation of arousal during social interaction.

Humans exposed to chronic or traumatic stress are at risk of developing pathological conditions such as depression or post-traumatic stress disorder (PTSD), which often negatively affect social interaction. Patients with depression, for example, show reduced interest in social activity, while patients with PTSD may show increased aggression. Some speculate that these symptoms relate to dysfunctional control of arousal by LC[1]. LC modulates neuronal response thresholds via noradrenalin (NA). In this way it regulates attention and arousal, and the response to behaviorally relevant stimuli[2].

Stress-induced dysfunction of the noradrenergic system is speculated to lead to chronic changes in the sensitivity of LC to its inputs. These changes in sensitivity could result from prolonged hyperactivation via the stress response system. For example, during acute stress, LC is activated by the corticotropin-releasing-factor (CRF) [3] which is released by stress responsive nuclei such as the central nucleus of the amygdala. Then, behavioral models of depression, like early maternal separation in rats, increase the tonic firing rates of LC neurons and reduces their sensitivity *in vitro*[4]. Finally, a single prolonged traumatic stress model of PTSD decreased spontaneous firing but increased stimulus-elicited activation in LC neurons in anaesthetized animals[5].

Causal links between LC hyperactivation and long-term changes in LC firing patterns have not been established. How such changes in arousal modulation impact behavior is unclear. I am specifically interested in

the impact of chronic social defeat on locus coeruleus activation. Chronic social defeat is traumatic to the submissive male and leads to social avoidance[8]. This behavioral adaptation is used as a model for depression.

I hypothesize that (1) chronic social stress affects the response of LC to social cues and during social interaction, and (2) chronic activation of LC is sufficient to induce behavioral symptoms of depression.

My approach is to measure the changes in LC firing patterns and courtship behavior in sexually experienced male mice by repeated social defeat or pharmaco-genetic hyperactivation of LC. In a control group I will suppress LC during social defeat treatments. I will measure LC firing rates and behavioral engagement in the freely behaving mice during exploration of social cues and during courtship before and after treatment.

Treatments: The test animals will experience repeated stress by social defeat. Each animal will be introduced into the home cage of a more aggressive male. After defeat, both mice will be kept in the same cage, separated by wire mesh for 24h. The test mice will be confronted with a different rival each day for a week. Pharmaco-genetic activation of LC will be induced by daily injections of CNO in mice that received viral transfection of excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs).

Controls: Next to an untreated control group, I will use mice infected with an inhibitory DREADD which will be activated during the social defeat treatments to test whether LC activation was necessary to develop behavioral pathologies.

DREADD: Transgenic mice of the TH-cre line co-express cre-recombinase protein with tyrosine-hydroxylase (TH). TH is essential for the biosynthesis of noradrenalin and dopamine and thus expressed in noradrenergic and dopaminergic neurons. Luckily, there are no other TH expressing areas in the vicinity of LC. We can therefore specifically target LC by local microinjection of cre-dependent vectors. These vectors will introduce excitatory or inhibitory DREADD into the noradrenergic neurons. DREADDs will then be activated by intraperitoneal injection of the DREADD agonist CNO. I already successfully targeted LC for cre-dependent viral infection with GFP.

Data acquisition: A 16-electrode probe (Innovative Neurophysiology) will be chronically implanted in LC of male mice. An integrated commercial multichannel data acquisition setup (Tucker-Davis, system 3) will be used to capture synchronized infrared video data, ultrasound audio data and extracellular neurophysiological data (figure 1).

Recording sessions: The sexually experienced male mice will be allowed to explore social cues consisting of urine samples from dominant males and females in estrus. These are assumed to elicit a LC response because they are behaviorally relevant. Female urine samples were reported to elicit vocalization in male mice. Odors will be placed in a small, open tube that will then be attached to the experimental arena from the outside in front of a small hole in the wall of the arena. The test mouse gains access from the inside when a sliding door is opened to uncover the hole. To measure LC activation in courtship, a female mouse in estrus will be released into the cage. 15 minutes are sufficient for the mice to show courtship and rejection, or acceptance and mat-

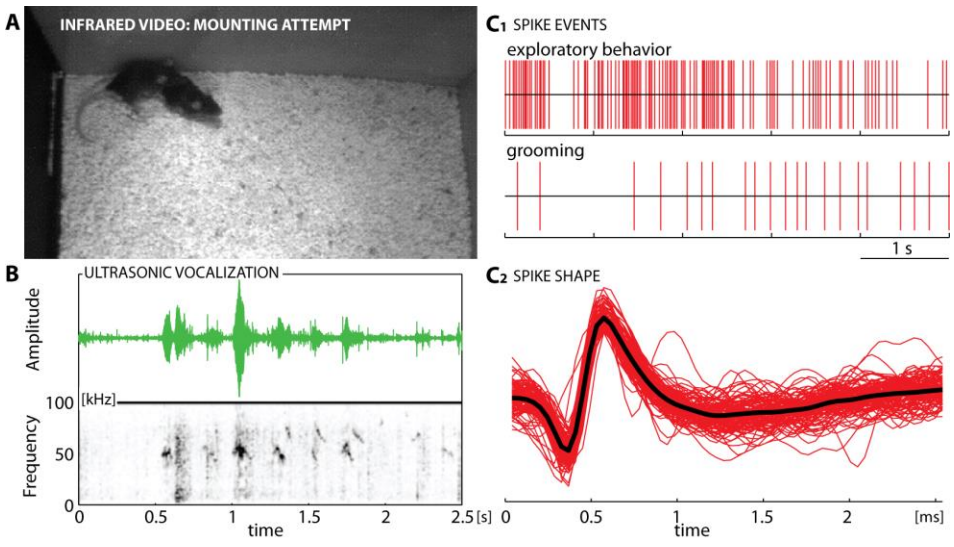

Figure 1 Behavior and electrophysiology in unrestrained behaving mice. A is a video frame taken during a mounting attempt. B depicts ultrasound vocalizations that occurred during the same mounting attempt. Upper panel shows amplitude over time, lower panel depicts a frequency spectrogram of the same data that indicates typical calls. C shows electrophysiological results from a different experiment in an awake and unrestrained behaving mouse. C1 shows two raster plots of spike events over time (5s total). Higher firing rates were detected during exploration than during grooming. C2 shows the shapes of the according spikes (red) and an average spike (black).

ing behavior.

Analysis: LC activation will be quantified by tonic and phasic firing rates as well as the frequency of bursts and overall action potential counts. Behavioral data will be analyzed quantitatively by motion parameters, different modes of courtship and the occurrence of vocalizations. Then, electrophysiological and behavioral data will be compared to find correlations between behavior and simultaneous activation of LC. The results will be compared between measurements taken before and after the treatment.

Outcomes: I expect in untreated mice that the isolated olfactory cues will induce burst firing in LC in response to female social cues. Urinary olfactory cues from female mice induce vocalizations in males, a behavioral indicator for arousal which should correlate with LC activation. For mice that underwent social defeat without pharmaco-genetical inhibition of LC, I expect overall decreased behavior and LC firing response to social cues, reflecting a decreased interest in social interaction. However, it is possible that the response to female cues is reduced while that to male cues is increased generally or specifically for the urine probes from their adversaries, reflecting increased sensitivity or anxiety. I expect that in mice that underwent pharmaco-genetical inhibition of LC during repeated social defeat, LC activation and behavior will be unaltered, as LC activation is supposed to be vital for the intensity of stress experience.
In social interaction I expect a very complex firing of LC. Nevertheless I will attempt to predict behavioral engagement from LC activation in the untreated group. In stressed mice however, the relationship between LC activation and behavior could change due to a changed sensitivity to noradrenalin in target brain areas, as suggested for chronic social defeat[10].

Pitfalls and Experimental alternatives: The proposed experiments are very ambitious and especially the multi-channel recordings from freely behaving animals are difficult. It is possible to perform the electrophysiological tests in a head fixed paradigm, instead. Such experiments are routinely conducted in our lab. An odor presentation apparatus is also in place that allows time controlled odor stimulation.

Further combination of chronic electrophysiological recordings with pharmaco-genetical manipulation might be too difficult to yield sufficient results in due time. In this case I would focus on a behavioral read-out, omitting the multi-channel electrophysiological recordings when manipulating LC activity. Comparison between the experiments in this case would be indirect but still informative about the relationship of LC firing and behavior.

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