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Intensity of anxiety is modified via complex integrative stress circuitries

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ABSTRACT

Escalation of anxious behavior while environmentally and socially relevant contextual events amplify the intensity of emotional response produces a testable gradient of anxiety shaped by integrative circuitries. Apprehension of the Stress-Alternatives Model apparatus (SAM) oval open field (OF) is measured by the active latency to escape, and is delayed by unfamiliarity with the passageway. Familiar OF escape is the least anxious behavior along the continuum, which can be reduced by anxiolytics such as icv neuropeptide S (NPS). Social aggression increases anxiousness in the SAM, reducing the number of mice willing to escape by 50%. The apprehension accompanying escape during social aggression is diminished by anxiolytics, such as exercise and corticotropin releasing-factor receptor 1 (CRF_1) antagonism, but exacerbated by anxiogenic treatment, like antagonism of α_2-adrenoreceptors. What is more, the anxiolytic CRF_1 and anxiogenic α_2-adrenoreceptor antagonists also modify behavioral phenotypes, with CRF_1 antagonism allowing escape by previously submissive animals, and α_2-adrenoreceptor antagonism hindering escape in mice that previously engaged in it. Gene expression of NPS and brain-derived neurotrophic factor (BDNF) in the central amygdala (CeA), as well as corticosterone secretion, increased concomitantly with the escalating anxious content of the mouse-specific anxiety continuum. The general trend of CeA NPS and BDNF expression suggested that NPS production was promoted by increasing anxiousness, and that BDNF synthesis was associated with learning about ever-more anxious conditions. The intensity gradient for anxious behavior resulting from varying contextual conditions may yield an improved conceptualization of the complexity of mechanisms producing the natural continuum of human anxious conditions, and potential therapies that arise therefrom.

1. Introduction

Anxiety covers a spectrum of related disorders affecting as much as 25% of the population, is highly comorbid with depression (Kessler et al., 2010; Reus et al., 2014), and is theoretically constructed along a fear/stress continuum spanning from apprehension to terror (Blanchard and Blanchard, 1989; Estes and Skinner, 1941; Freud, 1953). Recent clinical trials suggest that the predictive power of most single anxiety level/niche tests is low (Haller and Alicki, 2012). Many anxiolytic agents tested in clinical trials have been ineffective (Haller and Alicki, 2012), required a specific narrowing of the treatment group (Holsboer and Ising, 2010), or had adverse effects, sedation, and/or dependence liability (Mohler, 2012). We suggest that for greater translational salience, new models should contiguously examine multiple levels along a gradient of anxiety, and the contextual niches that make up the complex structure of real anxious behavior (Haller

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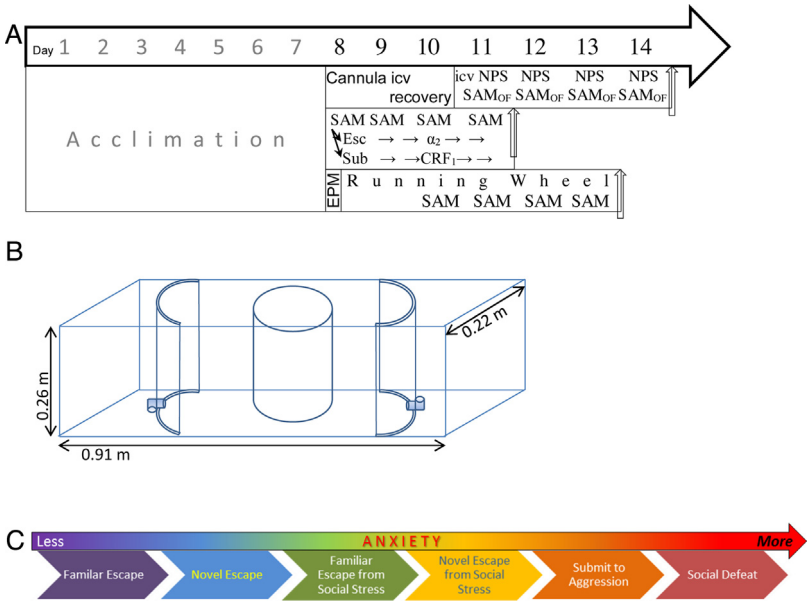

Fig. 1. (A) Timelines of the experimental protocols all begin with seven days of acclimation to cages, followed thereafter by anxiogenic or anxiolytic treatments. Interaction with SAM apparatus occurred over four days, and may have taken place in the presence (middle and bottom timelines) or absence (top) of a larger conspecific aggressor. (Top) For experiments in which the anxiolytic neuropeptide S (NPS) is delivered icv, intraventricular cannula placement was executed on day eight followed by recover until icv injection of NPS and interaction with the empty SAM apparatus of each of days eleven through fourteen to test for open field (OF) anxious behavior and escape. (Middle) Social anxiety and escape was tested by ip injection of the know anxiogenic α_2-adrenoreceptor antagonist yohimbine in escaping animals (determined on days 1 and 2 of SAM interaction; days 8 and 9 overall) or the anxiolytic CRF_1 antagonist antalarmin in submitting animals, just prior to day 3 and measuring latency to escape or inhibition of escape on days 3 and 4 of SAM social interactions (days 10 and 11 overall). (Bottom) For the voluntary exercise experiments, mice were tested for predisposition for anxious behavior on the EPM on day 8 and then provided a running wheel for 6 days. Social interactions in the SAM begin after 2 days of running, and continue for another 4 days (days 10–13 overall). The icv NPS experiment ended on day 14, the yohimbine/antalarmin experiments on day 11, and the running wheel experiments ended on day 13. (B) The SAM apparatus includes an open field (OF) arena that can be adjusted for size. The OF includes two escape routes, which lead to safe zones only accessible to small test mice. Test mice are added within the opaque cylindrical divider. Large aggressors are added outside the cylindrical divider. The divider is removed to allow social interaction. (C) The continuum or gradient of intensity of anxious behavior as revealed by the Stress-Alternatives Model.

and Alicki, 2012). We introduce a testable gradient of anxious behavior and hypothesize that an integrative machinery of stress and decision-making neurocircuitries produce this continuum and putative therapies.

The Stress-Alternatives Model (SAM) assesses anxious and depressive behaviors plus influences on decision-making (Smith et al., 2014), parsing active responses into contextual niches along an anxiety gradient. In the open field test (OF) rodents avoid the open center, which is reversed by anxiolytic drugs (Heredia et al., 2014). Mice in the SAM OF similarly avoid the center, but also actively choose to escape into an unknown chamber (Smith et al., 2014). Basic SAM escape experiments are modified by social aggression to increase the intensity (Koolhaas et al., 1997) of anxious behaviors and stress responsiveness (Smith et al., 2014) due to the uncontrollability and unpredictability of interactions (Koolhaas et al., 1998; Summers et al., 2005). Half the test animals remain submissively, exhibiting fear conditioning and increased corticosterone, and the rest escape. In SAM experiments using trout, rats, or hamsters, increasing social anxiety is accompanied by elevated plasma corticosterone concomitant with altered brain-derived neurotrophic factor (BDNF) and increased neuropeptide S (NPS) in the amygdala (Arendt et al., 2012; Robertson et al., 2015; Smith et al., 2014). As the SAM examines decision-making under socially stressful conditions by giving a choice of behavioral responses (escape or remain submissively), along with OF escape under non-social conditions (Smith et al., 2014), it has the fundamental advantage of examining a range of general and social anxious behaviors, as well as depressive behavior, allowing examination of the hypothetically complex range of circuit chemistry that is thought to produce progressively intense anxious behavior.

Neurocircuitries for stress, fear conditioning, decision-making, anxiety, and depression are highly interwoven, and include regions of the extended amygdalae and hippocampi, linked to periaqueductal gray, and controlled by executive regions of the cortex (Arendt et al., 2012; Herman and Cullinan, 1997; LeDoux et al., 1988; Li et al., 2013; Shin and Liberzon, 2010; Smith et al., 2014). Common to these circuitries, the central amygdala (CeA) is responsible for fearful/anxious signal transfer to other regions which modulate behavioral response, verified optogenetically (Tye et al., 2011). The intra-amygdalar fear/anxiety circuitry includes a glutamatergic pyramidal circuit (lateral = LA, basolateral = BLA) modified by GABAergic modulation and output (BLA, intercalated, CeA), both additionally modified by CRF, orexin (Orx), NPS, norepinephrine (NE), and serotonin (5-HT). There is strong evidence for modification of anxious behavior, and synergistic cross-talk among transmitter and neuromodulatory elements in this circuit (Arendt et al., 2014, 2013; Cannella et al., 2013; Jungling et al., 2008; Li et al., 2015; Orsini and Maren, 2012; Smith et al., 2014).

Our experiments were designed to investigate an intensity gradient of anxious behavior, and mechanisms that may produce and alleviate varying intensities of anxiety. We postulate that the neural machinery that produces progressively intense anxious behavior integrates numerous neuromodulatory elements of stress and decision-making neurocircuitries. Therefore, both anxiogenic and anxiolytic treatments, some intrinsic to SAM (novel escape route [NER], OF, social interaction and defeat), were included in these studies (Smith et al., 2014). Exogenous drug treatments and physiological enrichment (running wheel), which modify anxious state, were also included. We hypothesized that conditions that promote apprehension (OF, NER, social aggression, social defeat, yohimbine) would delay or inhibit escape from the SAM arena, depending on the intensity of those anxiogenic treatments. In contrast, we hypothesized that anxiolytic treatments (prior escape, running wheel, NPS, antalarmin) would promote escape behavior, and