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Using rat ultrasonic vocalization to study the neurobiology of emotion: from basic science to the development of novel therapeutics for affective disorders

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The use of ultrasonic vocalizations as an experimental tool for studying emotional states in rodents has led to an increased understanding of the basic science of affect as well as the development of novel diagnostics and therapeutics for the treatment of affective disorders. At the behavioral level, the rules that govern the generation of affective 'feeling' states are similar to those of the psychophysics of sensory perception. Emotions are elicited primarily in response to active social stimuli. A linear increase in affective response requires a logarithmic increase in stimulation and habituation of a given affective response allows for transition across the cycle of emotional/affective states (approach → consummatory phase → avoidance). At the neuronal level, the coordinated expression of affective responses in the medial prefrontal cortex is orchestrated by rhythmic activity, which is initiated and maintained by a variety of short-term and long-term synaptic plasticity processes. An objective measure of affective states may emerge from these psychophysical and neuronal properties of emotion. Enhancing synaptic plasticity with pharmacological agents that modulate NMDA receptor activity as well as IGFI receptor activity may have therapeutic potential for the treatment of affective disorders.

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Basic science of affect

Rat ultrasonic vocalizations as a measure of affective states

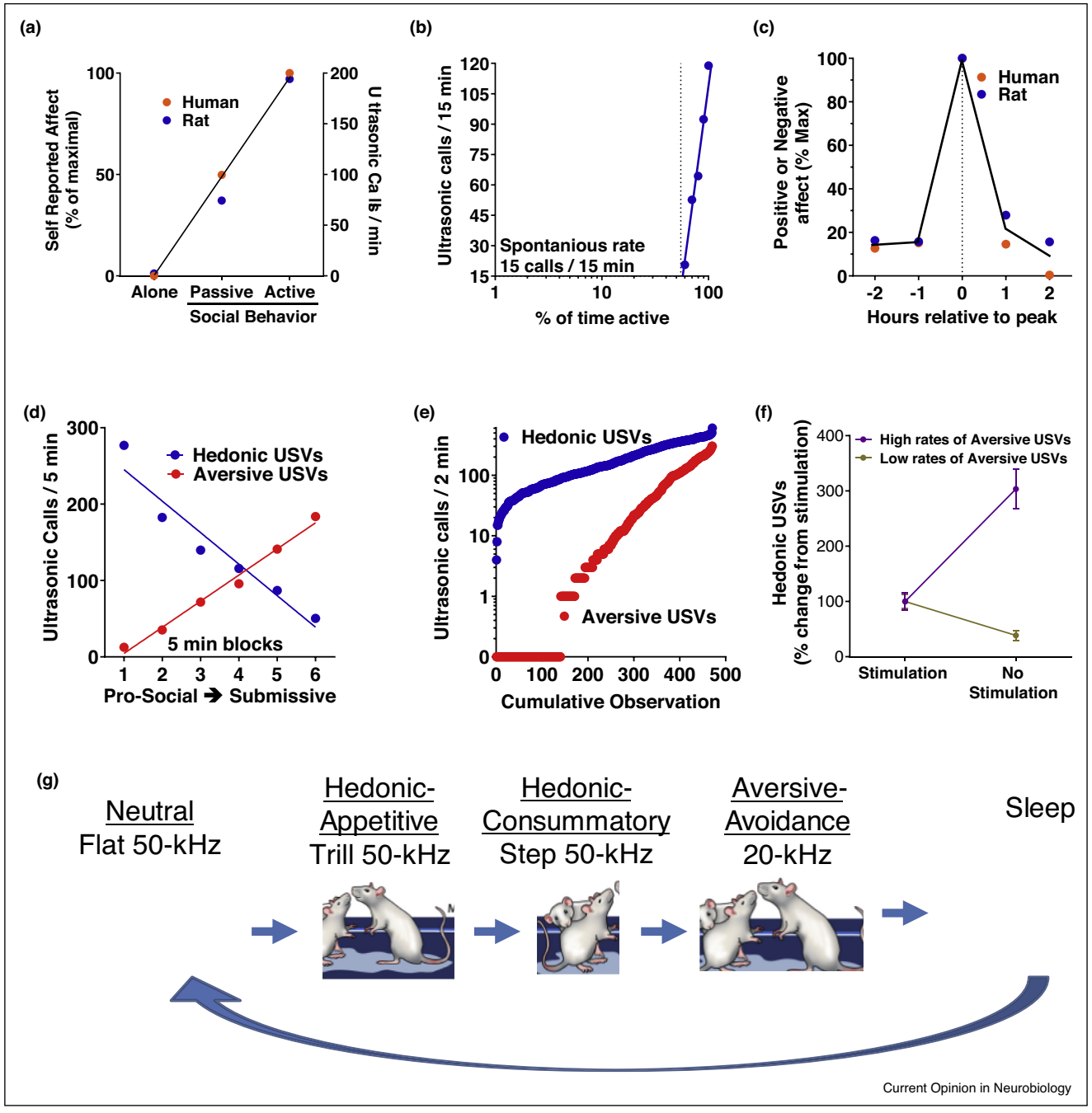
In this review we conceptualize emotion as 'sensory' feeling states elicited by social stimuli with the intensity determined by arousal and valence determined by the internal state of the organism. In this radio model of emotion (Figure 2), mood represents the tuner (valence), and emotion the volume (pulsatile change in volume). Affect combines both mood and emotion.

Emission of vocalizations is one of the fundamental behavioral manifestations of affective states in all vertebrates. Central pattern generators and motoneurons associated with emission and regulation of vocalizations are found in the evolutionary oldest parts of the brainstem at the junction with the spinal cord [1–3]. The control of the vocal system evolved very early in vertebrate evolution, and serves primarily a social function [2]. In rodents, ultrasonic vocalization (USV) evolved to coordinate mating, aggression and antipredator behavior. Emission of these calls represents emotional expression, mostly directed to conspecifics. Rat USVs have become a well-established measure of emotional/affective states in rats. Frequency modulated 50-kHz USVs (hedonic calls) reflect a positive affective state, whereas flat 22-kHz USVs (aversive calls) reflect a negative affective state [4,5]. These call categories have been consistently used as measures of an animal's emotional/affective state.

The new ethology of affect: psychophysical properties

The recent development of measures of affect in the everyday life of humans has revolutionized the study of emotion, and the rules that govern these 'feelings' are similar to the psychophysics of sensory perception. Using a psychophysical framework, the relevant 'stimulus' for eliciting an affective state is active social interaction, which is the best elicitor of self-reported affect in humans [6,7] and affective USVs in rats [8,9] (Figure 1a). The relationship between the degree of social interaction and affective state is log-linear, with a logarithmic increase in the amount of social interaction leading to a linear increase in affect. This relationship, which is best illustrated in the circadian rhythm of arousal and affect in humans [6,10] and in rat hedonic and aversive USVs [11]

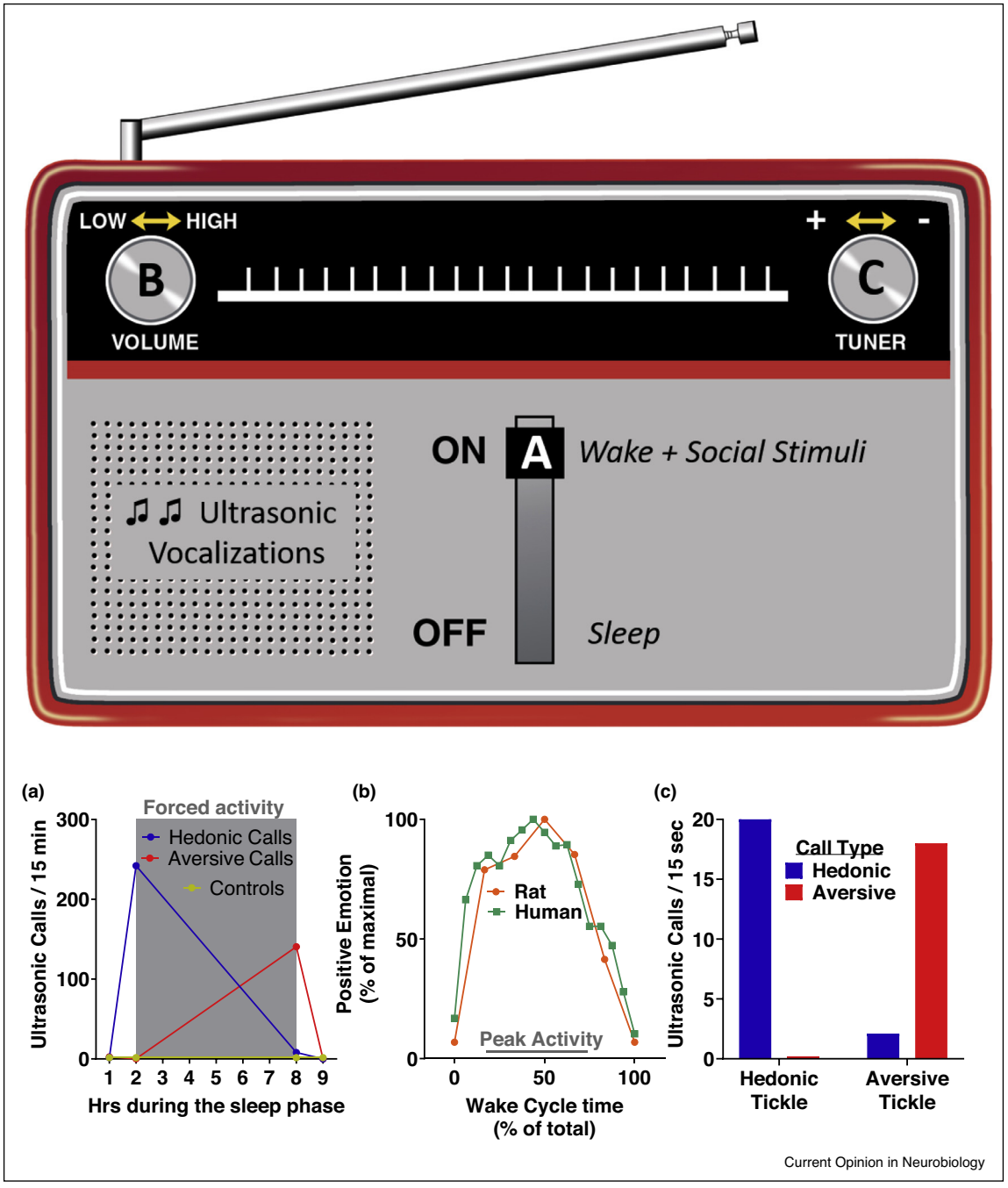
Figure 1



The psychophysics of affect. Affect is primarily induced by **(a)** active social interaction in both rats [8] and humans [6], in a manner that is log-linear in response to **(b)** arousal based on spontaneous homecage vocalization rates and activity levels [11] and **(c)** time in both rats [11] and humans [12]. The **(d)** habituation of a given affective response allows for the transition to a second affective response, which is controlled by **(e)** different thresholds for inducing different affective states and the **(f)** inhibitory effect of one affective state over another [8,13]. The sum of these properties leads to the cycle of affect **(g)**.

(Figure 1b), allows for a fine gradation of 'just noticeable differences' in affective state intensity in response to the intensity of the social interaction. This, in turn, provides information about social approach/maintenance/avoidance decision making. A similar log-linear relationship is seen in the temporal component of the emotional/affective state. Affective intensity increases logarithmically during the induction phase of the affective state and decreases logarithmically (~15 min half-life) during the decay phase in both rats and humans [11,12], as shown in

Figure 2



Experimental models for studying the radio Model of affect. **(a)** On/off switch can be studies with an automated tickling apparatus, in which quiescent socially housed rats can be induced to produce affective responses via forced activity/arousal. **(b)** The volume can be examined by studying the 24 hour rhythm of emotion in which peak affect occurs at peak activity/arousal at midcycle which is remarkably similar in both rats [11] and humans [10]. **(c)** The tuner can be studied by alternating between hedonic tickling and aversive tickling.

Figure 1c. The cycle of emotional/affective states may be illustrated in rats by recording the pattern of emission of their USVs: arousal → emission of appetitive trill 50-kHz USVs → consummatory step with 50-kHz USVs → emission of aversive 22-kHz USV → sleep (Figure 1g). This cycle is driven by the habituation of positive affect and subsequent disinhibition of negative affect across time in rats (Figure 1d), which is controlled by the threshold for inducing positive and negative affect (Figure 1c) and by the tonic inhibition of negative affect by positive affect

(Figure 1f) [8,13]. This is best illustrated with the Troxler effect/Cheshire cat visual illusion, in which the perception of the foreground image habituates revealing a background image.

Emotions are ultimately controlled by the internal state of the organism, although emotional states are based primarily on the processing of social sensory input. The same external stimulus, in this case tickling, that elicits hedonic 50-kHz USVs in normal young rats, elicits aversive 22-kHz USVs in chronically stressed rats or animals in chronic pain [14,15]. Current ongoing studies show that the amplitude of the emotional response (positive or negative) changes dramatically with the age of the animal. Additionally, the affective response to social stimuli are learned, albeit very quickly, across time [16,17].

Studies of emotional expression in humans via self-report as well as in rat using ultrasonic vocalizations show that the vast majority of the emotionally intense events are elicited by social stimuli [6,9]. We would classify non-social emotions as sensory pleasures (food and temperature regulation) which has a separate neurobiological substrate and has been well reviewed elsewhere [18].

The neuronal circuits of affect

The initiation of the emotional/affective process

The development of emotional/affective states in the mammalian brain occurs across all levels of the neuraxis, from deep brainstem to the neocortex. Classical experiments performed on cats in the first half of the 20st century demonstrated that the brainstem and hypothalamus are the anatomical sites generating complete and physiologically coordinated emotional/affective response, without any participation of higher brain structures such as the thalamus, septum, or cerebral cortex. These conclusive findings originated from two groups of investigators using decorticated preparations and intracerebral electrostimulation. Bard [19] summarized these pioneering studies by stating that "*there can be little doubt that both groups of investigators activated one or more specific brain-stem mechanisms that are welding together somatic and autonomic activities in such a way that a definite and unmistakable pattern of defensive, aggressive, or fearful behavior is produced*." (page 1190, [19]).

The primary location of the initiation of emotional/affective processes is below the hypothalamus in the brainstem. Ellison and Flynn demonstrated that surgical isolation of the cat hypothalamus, which severs all connections between the hypothalamus and the rest of the brain, did not prevent induction of the emotional/affective response that was elicited by deeper brainstem stimulation [20]. The fact that without stimulation these animals were inactive suggests that the hypothalamus is the lowest brain structure that integrates, organizes, and initiates emotional/affective behaviors, but is not the primary source of this process.

The ascending emotional arousal systems

As studied in rats, two ascending mesolimbic tegmental systems, which originate from the upper brainstem, provide input to many basal forebrain limbic regions and are involved in the generation of emotional arousal. These two systems are separate from the classical reticular activating system and cognitive arousal [21], as it was also recently concluded in the discussion on the relationship between cognitive and affective brain processes [22]. The activity of the ascending mesolimbic cholinergic system, which originates from a neuronal subpopulation of laterodorsal tegmental nucleus [21,23], generates aversive (negative) arousal that results in decrease and secession of activity (freezing or hiding), augmented attention, readiness for quick motor acts (escape or flight), and emission of 22 kHz ultrasonic vocalizations. While the activity of the ascending mesolimbic dopaminergic system, which originates from a neuronal subpopulation of the ventral tegmental area, generates appetitive (positive) arousal that results in increased motor activity, approach behavior, emission of 50-kHz vocalizations, and readiness to play in juvenile animals [21,24–26].

These two parallel emotional arousal systems evolved to prepare the animal for rapid and direct behavioral responses to emotional stimuli. Emotional arousal does not depend on one common arousal, but forms two valence-specific systems because they prepare the animal's response for two diametrically different outcomes. As such, these two ascending emotional arousal systems work in a mutually exclusive and antagonistic way [27].

The reticular core, including the laterodorsal tegmental nucleus and the ventral tegmental area, has direct ascending projections to many limbic structures as well as to the hypothalamus [28–30]. The hypothalamus is critical to the full development of the emotional/affective response, as well as to the integration of somatic, autonomic, and endocrine components of responses into patterns that take into consideration internal and external environments (McLeary and Moore, 1965). While emotional arousal is initiated within milliseconds, a fully-blown emotional state takes several minutes to develop, as observed on polygraphic recordings in cats with pharmacologically induced emotional responses [31].

Closer inspection of pharmacologically induced emotional responses from the hypothalamus or other limbic regions reveals that these fully-developed, clear affective states are lacking the complexity of an integrated emotional expression. Microinjections of amphetamine in the nucleus accumbens in rats are perhaps the best elicitors of emotional responses with hedonic 50-kHz USVs. However, these calls are exhibited in the absence of social

stimuli and the responses are not directed anywhere. Eventually a suppression of social behavior is seen when, for instance, two amphetamine-treated rats are placed together in a cage. Thus, an emotional response that is fully integrated with the environment and with calling in response to social stimulation and with coordination of social behavior is not seen. The same was observed with carbachol-induced aversive responses in cats with emission of growling vocalizations [32,33]. After injection, an emotional/affective response was developing, but the animals were not able to find the ethologically-relevant source of this state. They began cautiously, but increasingly, investigating the environment, with documented increases in the number of head turns ('looking around' movements) and eye movements [32,34]. The description of this behavior stated that the set of behavioral manifestations "*gave the cats' behavior an air of 'cowardly looking around' as if the animals 'were expecting some danger' or were 'seeking the source of danger'*" (page 20, [32]). The responses were not adequate to the environment and not directed.

The medial prefrontal cortex as the 'conductor' of affect

The 'conductor' property of the medial prefrontal cortex is well illustrated by the results of electrical brain stimulation-induced emotional behavior. The most extensive study of human emotional response to deep brain stimulation to date was conducted by Mayberg *et al.* [35], using an optimized protocol for eliciting stimulation-induced affective responses. In their study, medial prefrontal cortex stimulation produced a well-integrated positive affective state, in which a subject (#7) smiles and self-reports '*I felt like laughing; I feel good*' as well as mentioning about goal-directed social behavior '*I would be walking my dog.*' Using a similar protocol in rats, electrical brain stimulation of the medial prefrontal cortex also induced a coordinated emotional response with hedonic 50-kHz USVs, along with playful joy jumps (Freudensprung) and increased social behavior [36]. However, electrical brain stimulation of the mPFC can also evoke aversive 22-kHz USVs and lesion in the mPFC decreases or abolishes aversive 22-kHz calls [37,38], suggesting the dependency on the stimulation frequency and subregions in the mPFC for inducing either positive or negative affect. In contrast, subcortical stimulation elicited only coordinated components of the fully expressed emotional response, and often induced compulsive self-stimulation behavior in both humans [39,40] and rats [36]. Therefore, the medial prefrontal cortex appears to direct and coordinate the expression of individual components of an emotional/affective response generated subcortically.

The medial prefrontal cortex orchestrates the subcortically generated emotional behavior by synchronized oscillatory mechanisms. In rats, hedonic USVs are associated with theta and aversive USVs with delta EEG oscillations [41]. These oscillations have been well studied in both humans and rats, usually in the context of sleep/wake EEG studies, and are associated with active wake/exploration for theta and drowsiness/sleep for delta rhythms as measured in the hippocampus [42,43] and recently by our group in the MPFC as an unpublished observation. Similarly, hedonic 50-kHz USVs at the biological maximal rate occur at ~6 per second (theta) and aversive 22-kHz USVs at ~2 per second (delta). Sniffing rates during hedonic calls and slow and forceful breathing during aversive calls also follow this same theta/delta rate [44]. Electrical brain stimulation that unconditionally induces sniffing behavior (theta) supports self-stimulation, whereas unconditional slow breathing (delta) is aversive to the animal [45].

The radio model of affective behavior

A relevant affective/social stimulus gates arousal/drive levels to engage the emotional system. Different affects have different thresholds for elicitation, and there is a progression through a given set of affective states that completes the appetitive/consummatory/satiety cycle of motivated behavior. The regulation of emotional/affective states in natural circadian cycles may be compared to the function of a radio set with its regulations as on-off switch, the volume, and the tuner.

It is also important to note that Non-social stimuli are also able to induce high rates of ultrasonic vocations, with forebrain microinjections of amphetamine and carbachol eliciting high rates of 50-kHz and 22-kHz USVs respectively [4,9]. Air puff to the nape of the neck as well foot shock can also elicit high rates of aversive calls [21]. However, in terms of naturalistic stimuli, social stimuli are by far the best elicitor of USVs, with the anticipation of food reward and frustrative food non-reward only eliciting modest rates of 50-kHz and 22-kHz USVs respectively and under very specific experimental conditions [46].

The on-off switch. In rodent experiments, the combination of emotional arousal and social stimuli are both necessary and sufficient to elicit robust rates of affective vocalizations. In the last 20 years of 'tickling' research, nearly all (if not all) rats showed emission of at least some hedonic or aversive USVs in response to tickling stimuli, with the tickling providing both the social and arousal components. Rat tickling resembles natural rough-and-tumble play of young rats, and is the most effective tool for inducing emotional/affective changes with robust vocalizations [9]. Recently, we developed an automated protocol for tickling in which group-housed rats are forced to move and interact with a constantly rotating rod (Figure 2a). These experiments occur in the animals' home cage and start during the middle of the light (sleep) phase of the circadian cycle, in which the animals are not vocalizing (social stimuli without arousal). Once the rotating rod is activated, the animals vocalize for

the duration of the time that the rod is activated (social stimuli + arousal).

The volume. Emotional/affective states show a clear deprivation-induced function in which affective drive builds up over deprivation and is released during social interaction. Thus, the amount of emotional arousal exhibited is proportional to the social deprivation. Traditionally, this was accomplished by social isolation and reunification. Using our protocol, 24 hour of deprivation is enough to induce a maximal bout of emotional/affective arousal 'on demand.' However, continuous home cage recording of USVs can accomplish this same effect, without the need for social isolation or experimental handling. During lights on, the rats primarily sleep and show little vocalizations, whereas as soon as the lights turn off, they show an increase in emission of USVs, which decay across time in the dark [11]. This protocol allows for the accumulation and release of emotional/affective arousal (the volume of the response) to be studied naturalistically (Figure 2b).

The tuner. The internal state of the organism and not the stimulus itself controls which affective state is exhibited. A good example of this is the tickling response in rats that underwent a chronic unpredictable stress paradigm for 21 days versus control rats. Whereas the control rats exhibited almost exclusively hedonic calls, rats subjected to the chronic unpredictable stress emitted aversive calls to the exact same stimulus [14]. In a more ethological setting, the shift from hedonic to aversive calls occurs across time during a social interaction, which naturally transitions animals from initiation and maintenance to termination of social behavior. In play and aggression studies, the termination period is associated with greater and longer pinning behavior and bites during the play. This can be modeled by using a modified version of the tickling test in which after the standard stimuli, the rats are pinched at the nape of the neck and gently shaken for 1 s; this is repeated every 5–15 s until aversive calls are exhibited. Alternatively, this can be modeled in an automatic tickle protocol using a longer duration of stimulation (15 s) in which 1 s of shaking while holding the nape of the neck is followed by 14 s of standard tickling, with a 15 s intertrial interval (Trail block 1–9; see Figure 2c). Thus, the transitions from positive-to-negative affective state, negative-to-positive state, and the suppressive power of one affective state over another can be examined in a rapid, 3 min assay.

Translational implications

Objective measures of affective states

Non-invasive EEG methods may be able to measure affective state, based on our model of affect. Affective states are driven by active social stimulation and arousal, and these states are generated by medial prefrontal cortex oscillations. Thus, it may be possible to predict affective states by measuring medial prefrontal cortex EEG activity during active social interactions and measure the daily rhythm of affect via EEG activity in concert with actigraphy.

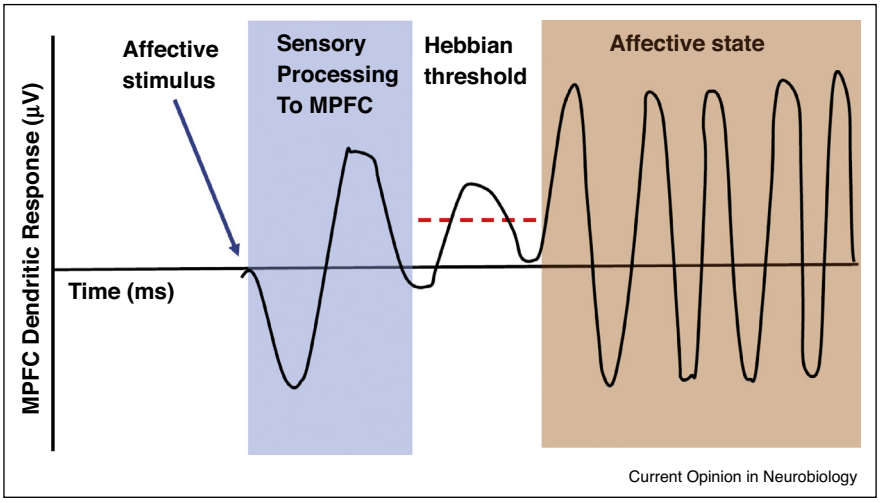
Sensory evoked EEG responses may be able to measure the individual neuronal circuits that generate affective states. Based on the model, an emotional response to affective sensory stimuli should first trigger an affective response, as measured by a change in dendritic potential, and lead to a sustained emotional response, as measured by EEG oscillations (Figure 3). Therefore, for a given emotional stimulus, a sensory evoked response in the medial prefrontal cortex may be able to measure the threshold for eliciting an affective state, whereas an evoked oscillatory behavior may be able to predict the strength of the affective response. Habituation to the sensory evoked response may measure affective habituation. The affective setpoint of the subject may be measured by the ability of the medial prefrontal cortex to entrain delta or theta sensory stimuli.

Modulation of affective synaptic plasticity as therapeutics for affective disorders

While decades of research have shown that monoamine modulation of the striatum and hypothalamus robustly elicit emotional responses in animals, these responses are fragmentary in nature and do not represent a fully ethologically integrated emotional response (as summarized above). A research program was undertaken to uncover the molecular substrates of positive affect in a relevant brain region (prefrontal cortex) and a relevant emotional eliciting stimulus (rough-and-tumble play) at the level of gene expression [47]. These studies revealed that the key modulators of synaptic plasticity in the medial prefrontal cortex (i.e. NMDA receptors, insulin-like growth factor, and likely others to be determined) were specifically upregulated by positive affect, and that direct pharmacological modulation of these targets enhanced a fully integrated positive emotional response [48–50].

Magnetic or electrical stimulation of the medial prefrontal cortex at theta/alpha frequencies has been shown to induce antidepressant effects in humans by entraining the medial prefrontal cortex at these rhythms [51]. Repeated transcranial magnetic stimulation of the frontal cortex at a frequency of 7 Hz produced robust antidepressant responses in humans across multiple clinical trials, and induced EEG entrainment. Likewise, electrical deep brain stimulation of the medial prefrontal cortex at 6–7 Hz also induced profound antidepressant effects in humans across multiple studies [35]. These antidepressant effects, therefore, may be due to stimulation of a positive affective state by the medial prefrontal cortex by entraining theta/alpha EEG oscillations, thereby suppressing a negative affective state and augmenting a positive affective state.

Figure 3



Neuronal model of rhythms of affective states. The affective value of stimuli is detected by the medial prefrontal cortex (MPFC). At a given threshold, an affective state is generated by inducing rhythmic behavior in the MPFC that coordinated the subcortical generation of affective state.

Inducing changes in long term synaptic plasticity by the induction of seizures with electroconvulsive shock therapy is perhaps the most effective antidepressant to date. Clinical protocols state that electroconvulsive treatment should induce seizures, and that repeated seizures and electroconvulsive sessions across time are necessary for optimal antidepressant response [52]. In animal studies, long term potentiation (LTP) induced by high frequency stimulation (100 Hz) performed *in vivo* in the medial prefrontal cortex induces seizures at relevant stimulation intensities, and repeated high frequency stimulation sessions are required to optimize the affective response of MPFC electrical stimulation [53]. In addition, spacing out the high frequency tetanus is optimal for inducing long lasting synaptic plasticity. Thus, it can be hypothesized that electroconvulsive shock therapy may be altering the positive/negative affective setpoint in the medial prefrontal cortex.

Synaptic plasticity can be modulated pharmacologically via multiple unique mechanisms. The induction of LTP requires NMDA receptor activation during the tetanus, and NMDA receptor-activation facilitates this form of LTP. Drugs that positively modulate NMDA receptors and enhance prefrontal cortex synaptic plasticity in humans have also produced antidepressant effects in clinical trials [54]. While LTP is induced by NMDA 'receptors, it is maintained by increasing mature dendritic spines that maintain Hebbian plasticity. IGFI (insulin-growth-factor-I is an FDA-approved growth factor therapeutic that enhances synaptic plasticity without the need for tetanus by enhancing mature dendritic spine formation. IGFI also has been shown to have antidepressant effects in clinical trials. IGFBP2 (IGF binding protein 2) has greater potency and a unique mechanism of action as compared to IGFI, and IGFBP2-based therapeutics may have therapeutic potential for the treatment of affective disorders [55].

Summary

The neurobiology of emotional 'feeling' states is strikingly similar to that of the neurobiology of touch, hearing, and seeing, with the same psychophysical properties and cortical hub for generating the fully integrated 'feeling' state. Positive affective states appear to be generated by theta/alpha oscillations and negative affective states by delta oscillations in the medial prefrontal cortex. The switch between negative and positive affect along with the threshold for eliciting an emotional state is mediated by an LTP-like synaptic plasticity processes in the medial prefrontal cortex. Theta/delta EEG power in the medial prefrontal cortex in response to affective stimuli may serve as an objective measure of affect with diagnostic value for affective disorders. The development of drugs that target synaptic plasticity in the medial prefrontal cortex may be useful therapeutics for affective disorders.

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