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Review

Frequency-modulated 50 kHz ultrasonic vocalizations a tool for uncovering the molecular substrates of positive affect

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ABSTRACT

The evidence that frequency modulated (FM) 50 kHz ultrasonic vocalizations (USVs) reflect a positive emotional state in rats is reviewed. Positive emotional states in humans are measured by facial-vocal displays (e.g., Duchenne smiling and laughter), approach behavior, and subjective self-report of feeling states. In laboratory animals, only facial-vocal displays, along with approach behavior, can be measured. FM 50 kHz USVs are uniquely elevated by hedonic stimuli and suppressed by aversive stimuli. Rates of FM 50 kHz USVs are positively correlated to the rewarding value of the eliciting stimulus. Additionally, playbacks of these vocalizations are rewarding. The neural and pharmacological substrates of 50 kHz USVs are consistent with those of human positive affective states. By experimentally eliciting FM 50 kHz USVs, the novel molecular underpinning of positive affect can be elucidated and may be similar to those in humans. In humans, positive emotional states confer resilience to depression and anxiety, as well as promote overall health. Using rough-and-tumble play induced hedonic USVs, we have identified that insulin like growth factor I and NMDA receptor 2B subunit as playing a functional role in positive affective states. From this research, we have developed a promising new class of antidepressants that is entering phase II clinical trials for the treatment of depression.

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1. Positive affective states in humans

1.1. Measuring positive affective states in humans

In humans, socializing with friends or romantic partners elicits the highest levels of positive affect state (Csikszentmihalyi and Hunter, 2003; Kahneman and Krueger, 2006; Stone et al., 2006). Experimental studies that elicit positive affective states generally

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use social positive affective stimuli (i.e., positive feedback, giving a small gift, or watching a video tape eliciting positive affective state). Positive affective states that are elicited in an experimental setting by these social stimuli have been shown to increase gregariousness, optimism, and openness to new experiences (Lyubomirsky et al., 2005; Fredrickson, 2004).

A functional distinction can be made between positive prosocial affective states primarily associated with subjective well-being and consummatory pleasures. Experimental study of nonsocial hedonic stimuli (i.e., food or regulatory thermal stimulus) showed that these pleasures function primarily to maintain homeostasis. For example, a warm stimulus would be experienced as pleasurable by a cold individual, with the magnitude of the pleasure being proportional to the ability of the stimulus to return the body to homeostatic conditions (Cabanac, 1971). This emotionally driven change in sensation associated with a return to homeostasis is referred to as sensory alliesthesia (Cabanac, 1971, 1992).

Positive affective states, as studied longitudinally in humans, confer resilience to depression and anxiety and lead to an increase in overall health and a decrease in mortality from all causes (Lyubomirsky et al., 2005). The psychological and physical health benefits of positive affective states appear to be mediated through increased resilience, defined as continued global functioning despite the presence of stressors. For example, following a major life stressor individuals exhibiting greater resilience are less likely to develop psychological disorders such as anxiety or depression (Fredrickson et al., 2003). Longitudinal studies also showed that positive affective states precede the health benefit effect of positive affect (Lyubomirsky et al., 2005). Therefore, positive affect is not simply a secondary consequence of overall good health. Major positive and negative life events having little long-term effect on these states (Lykken and Tellegen, 1996). Conversely, individuals who have low levels of positive affective states are at greater risk of developing anxiety disorders, depression, and global health problems (Lyubomirsky et al., 2005). Interventions that increase positive affective states have been shown to reduce levels of depression and anxiety (Duckworth et al., 2005).

At the present time, there is a growing positive psychology movement that is impacting how we understand human aspirations for a better life as well as animal well-being (for recent reviews, see McMillan, 2005; Sheldon et al., in press). Affective neuroscience can contribute a deeper neuroscientific understanding of how the various emotions, including positive ones, are organized in the brain (Panksepp, 1998). Without a neuroscientific understanding of our diverse affective states, both positive and negative, our approach to the treatment of depression and anxiety will remain incomplete (Panksepp, in press).

1.2. Neurobiology of positive affective states in the human brain

The primary neuroanatomical underpinnings of positive emotional states are associated with the ascending mesolimbic dopamine system and have relied primarily on correlational brain imaging studies (i.e., functional Magnetic Resonance Imaging or Positron Emission Tomography) and the direct elicitation of positive affective states through drug administration or electrical brain stimulation. Brain imaging studies using recall of positive affective memories (Damasio et al., 2000), listening to positive music (Blood and Zatorre, 2001), male orgasm (Holstege et al., 2003), and positive anticipation of monetary reward (Knutson et al., 2001), all have been shown to activate aspects of the ascending mesolimbic dopamine system that includes the ventral tegmental area, nucleus accumbens, medial prefrontal and orbitalfrontal cortices (Burgdorf and Panksepp, 2006). The euphoric effects of intravenous amphetamine have been shown to be directly related to dopamine activation in the nucleus accumbens (Drevets et al., 2001; Oswald

et al., 2005). Direct electrical brain stimulation of the accumbens has been shown to elicit Duchenne laughter and self-report of positive affect (Okun et al., 2004). Patients given the opportunity to self-administer electrical stimulation to the nucleus accumbens (then called the nucleus accumbens septi as a ventral extension of the lateral septum), or to an area at or near the ventral tegmental area, repeatedly self-administered this stimulation and reported that the stimulation elicited a positive affective state (Heath, 1963, 1972).

It is important to note that this type of emotional positive affect is distinctly different than the pleasures of sensory affects. The feeling is more one of eager anticipation, enthusiasm and euphoria rather than discrete pleasures such as those evoked by sensory stimulation induced by food, massage or orgasm. The name we chose to help reflect the concurrent behavioral and psychological functions of positively valenced appetitive behavior was EXPECTANCY and SEEKING (Panksepp, 1981, 1982, 1998), which is quite similar to the concept of 'wanting' which is thought to mediate 'incentive salience' (Robinson and Berridge, 2000). However, there is a substantial difference between 'incentive salience', which is fundamentally a sensory-perceptual process, and SEEKING, which is that (since each emotional system performs sensory-perceptual gating functions), as well as an integrated motor-action process (a coherent emotional response that allows organisms to pursue all of the resources needed for survival) (Panksepp and Moskal, 2008). The affect of SEEKING is tightly linked to the primary-process instinctual-action aspects of an organism and to the sensory inputs that the system harvests.

1.3. Molecular underpinnings of positive affective states

The molecular mechanisms that are involved in the regulation of positive affective states are largely unknown. The best understood mechanisms describe brain dopamine functions in appetitive motivations and endogenous brain opioids in mediating various sensory pleasures and bodily satisfactions, including social rewards (Burgdorf and Panksepp, 2001; Panksepp, 1981). Another example of a molecular mechanism involved in the regulation of positive affective states are endogenous cannabinoids, which modulate many emotional processes (Moreiram and Lutz, 2008), including the modulation of sensory pleasures as well as social ones such as physical play (Trezza et al., 2010). In order to develop a more comprehensive affective neuroscience strategy for identification of new hedonic pathways, we need to consider the criteria that would allow us to study molecular processes in animal models.

In order to establish a causal link between a molecular mechanism associated with positive affect, the following conditions must be fulfilled: (1) concentrations of key molecules associated with the mechanism under investigation must be significantly altered in critical brain regions following positive affective stimuli; (2) these molecular changes will change in the opposite direction or not change significantly following presentation of negative affective stimuli; (3) direct injection of the target molecules or agonists will produce a positive affective state; (4) and, pharmacological antagonism of the key molecules will decrease positive affective states. Thus far, no molecular mechanism has been characterized could meet all of these four criteria.

Endogenous opioids acting on μ -receptor s (endomorphins, met-enkephalin, and β -endorphin) and dopamine have been the most extensively examined (reviewed in Burgdorf and Panksepp, 2006). μ (μ)-opiate and dopamine levels in the mesolimbic positive affect circuit have been found to be positively correlated with the euphoric effect of exercise and amphetamine, respectively (Boecker et al., 2008; Drevets et al., 2001). Intravenous administration of μ -opiate and dopamine agonists produced positive affective states in humans (Drevets et al., 2001; Zacny et al., 1994). μ (μ -

Table 1
Non-affective hypotheses of 50 kHz USVs emission with their rebuttal.

I. 50 kHz USVs are an artifact of locomotor activity-induced thoracic compressions (Blumberg, 1992).	Only 10% of 50 kHz were coincident with thoracic compressions, and could be dissociated from locomotion (Panksepp and Burgdorf, 2003).
II. 50 kHz USVs are a non-affective contact call (Schwartz et al., 2007) interactions (Burgdorf et al., 2008).	Flat 50 kHz calls appear to be a contact call, occurring at the highest rates during non-positive affective social interactions. However, FM 50 kHz calls appeared to be selective for positive affective social.
III. 50 kHz calls are evident during aggression (Berridge, 2003).	50 kHz calls occur primarily before the onset of aggression, and the vast majority the 50-kHz calls were of the non-affective flat variety (Panksepp and Burgdorf, 2003; Burgdorf et al., 2008)
IV. 50 kHz calls reflect a non-positive affective "wanting" state (Schwartz et al., 2007).	50 kHz USVs were increased in the anticipation of delivered reward, which in humans has been shown to elicit a positive affective state (Knutson et al., 2001). However, during extinction bursts or "frustrative non-reward" such appetitive behavior decreased rates of 50 kHz calls and increased rates of aversive 22 kHz calls (Burgdorf et al., 2000) "frustrative non-reward" such appetitive behavior decreased rates of 50 kHz calls and increased rates of aversive 22 kHz calls (Burgdorf et al., 2000)
V. Adult and infant rat ultrasonic calls reflect a state of high arousal that is not specific to positive affective states (Bell, 1974).	Highly arousing aversive stimuli such as predatory odor, foot shock, and bright light, decrease rates of 50 kHz calls, whereas rewarding stimuli increase rates of 50 kHz calls (Knutson et al., 2002a,b)

opiate antagonists has been shown to blunt the positive affective state elicited by exercise and alcohol (Janal et al., 1984; Davidson et al., 1999), and dopamine antagonists decreased positive affective states associated with psychostimulants (Jönsson et al., 1971; Newton et al., 2001; Romach et al., 1999) and could produce a state of dysphoria (Voruganti et al., 2001). However, aversive stimuli also increase μ -opiate and dopamine levels in the nucleus accumbens (Tidey and Miczek, 1996; Marinelli et al., 2004). Therefore, the μ -opiate and dopamine systems are not completely specific to positive emotions. Part of their hedonic action may be due to alleviation of negative feelings.

2. Measuring positive affective states in laboratory animals

In order to establish that an animal behavior reflects a positive affective state, several criteria must be met. In humans, positive affective states are measured primarily via subjective self-report and behaviorally by facial/vocal displays such as felt- or Duchenne-smiling (Ekman et al., 1990). Therefore, in laboratory animal experiments, where we can rely only on empirical observations with no possibility of semantic reports of subjective states, a positive affective state should be expressed as facial or vocal displays with the predicted changes in approach/avoidance behaviors, and especially by certain central states in animals, as provoked by local brain stimulation, whether chemical or electrical (Panksepp, 1998; Ikemoto, 2010) to serve as rewards in various learning tasks. In humans, positive affective states are elicited primarily by rewarding social interaction, food, and exercise, and are decreased by negative affective stimuli (Csikszentmihalyi and Hunter, 2003; Kahneman and Krueger, 2006; Stone et al., 2006). Therefore, in laboratory animals, the same categories of positive affective (appetitive) stimuli should increase the facial/vocal displays and aversive stimuli should decrease them. Finally, what is known about the neurobiological mechanisms of the facial/vocal displays in animals should be consistent with the neurobiological mechanisms of human positive affective states. To date, only two such animal behaviors meet all of these criteria; emission of ultrasonic vocalizations (USVs) that are discussed below, and hedonic taste reactivity reviewed by Berridge et al. (2008). In addition, from a more strictly neuroscientific perspective, the gold standard that direct stimulation of certain brain networks should have rewarding properties (Panksepp, 1998), has been well documented ever since the work of Olds and Milner (1954), validated psychologically with human brain stimulation studies (Heath, 1972, also see Volker et al., this issue).

We seek to develop a vocal output measure of positive affective states, such as the way that screaming expresses pain-perception in animals. Research involving laboratory rodents expressing emotional vocalizations in the ultrasonic range has been proposed as potential non-semantic 'self-report' measures when animals are in positive affective state (Brudzynski, 2007; Knutson et al., 2002a,b; Panksepp et al., 2002).

2.1. 50 kHz social vocalizations in rats

Fifty kilohertz ultrasonic vocalizations (50 kHz USVs) have been shown to reflect a positive affective state in rats, especially the frequency-modulated variety. The less complex "flat" variety, may be a social-exploration/contact signaling mechanism that is less indicative of positive affect. Rewarding social interactions (i.e., mating and rough-and-tumble play in juveniles), anticipation of food, and action of euphorogenic drugs of abuse increased number of emitted 50 kHz USVs (Burgdorf et al., 2000, 2001a, 2007, 2008; Panksepp and Burgdorf, 2000), whereas aversive stimuli such as social defeat, frustrative non-rewarding situations, sickness-inducing doses of lithium chloride, and foot shock all decreased the number of 50 kHz USVs (Burgdorf et al., 2000, 2001b, 2008). The rewarding value of the stimuli eliciting positive affective states was positively correlated with the rates of 50 kHz USVs elicited by positive social, drug, and electrical brain stimulation rewards (Burgdorf et al., 2007, 2008). μ (μ) opiate and dopamine agonists, as well as electrical brain stimulation of the mesolimbic dopamine system, also increased rates of 50 kHz USVs in rats (Burgdorf et al., 2000, 2007).

Additionally, alternative non-hedonic interpretations of the emission of 50 kHz USVs (e.g., non-positively valenced arousal, non-positively valenced seeking behavior, or non-affective social contacts) are not supported by the available experimental data (for details, see Table 1). Further, this measure has already proved useful in studies of drug addiction characterizing resilient and non-resilient rats in studies of the neuroanatomical regions that are impacted most by stressors that promoted depression (Kanarik et al., in press; Mällo et al., 2009). Indeed, tickling of rats, a way to quickly evoke positive social affect in rats (Burgdorf and Panksepp, 2006; Panksepp and Burgdorf, 2000).

2.2. 22 kHz aversive vocalizations in adult rats and isolation calls (35–40 kHz) in infants

Adult 22 kHz USVs and infant isolation calls (35–40 kHz) may represent a negative emotional state associated with human anxiety.

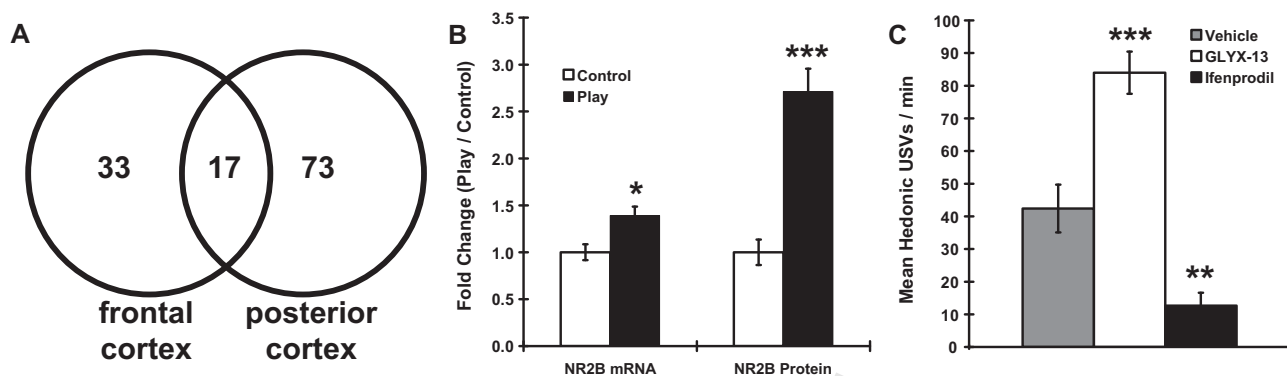


Fig. 1. The NMDA NR2B subunit plays a functional role in play-induced hedonic 50-kHz USVs. (A) Venn diagram of significantly changed gene by oligonucleotide microarray in the frontal and posterior cortex 6 h after rough-and-tumble play. The NMDA receptor gene family was significantly upregulated among the 17 shared genes, and not in the 33 or 73 genes alter only in the frontal or posterior cortex respectively. (B) The NR2B subunit mRNA and protein levels were upregulated in the frontal and posterior cortex as determined by qrtPCR and western blot. (C) Play induced Hedonic USVs were increased by an agonist dose of the NR2B preferring glycine site partial agonist GLYX-13 (1 mg/kg), and decreased by the NR2B antagonist ifenprodil (3 mg/kg). * $P < .05$, ** $P < .01$ and *** $P < .0001$.

ety and/or depressive states (e.g., aversive facial expressions such as crying, and behavioral inhibition (Knutson et al., 2002a,b)). Despite significant sonographic differences between these adult and infant vocalizations, both of them share similar characteristics in aversive and dangerous situations. In humans, these affective states are often elicited by social loss and anticipation of perceived threats (Knutson et al., 2002a,b). In rats, infant isolation 35–40 kHz USVs are best elicited by separating the pup from the mother. 22 kHz USVs are best elicited by social defeat and the presence of a predator (Blanchard and Blanchard, 1989; Brunelli and Hofer, 2007; Panksepp et al., 2007). Emission of 22 kHz USVs is strongly related to avoidance behavior and freezing during social defeat testing (Panksepp et al., 2007). Environments paired with drugs causing aversive states also elicit 22 kHz USVs. Rates of emitted calls are positively correlated with drug-induced conditioned place avoidance (Burgdorf et al., 2001b). Anxiolytic benzodiazepines and antidepressants reduce rates of 22 kHz calls and 35–40 kHz USVs (Carden and Hofer, 1990; Covington and Miczek, 2003).

Using social defeat as a method to elicit negative emotional states associated with 22 kHz USVs, we conducted a transcriptomic analysis of gene expression in the periaqueductal gray, one of the regions found to be critical for the generation of negative affect and 22 kHz USVs in rats (Kroes et al., 2007). These studies revealed that mRNA expression of genes associated with acetylcholine metabolism and receptor function was altered in the PAG following social defeat. This finding is consistent with the previously reported role of tegmental cholinergic system in the generation of 22 kHz USVs (Brudzynski, 2001). Carbachol has been shown to be the best elicitor of aversive vocalizations in both rats, cats, and squirrel monkeys (Brudzynski, 2007; Lu and Jürgen, 1993). Recent studies in humans demonstrated that depressed patients have altered cholinergic transmission (Wang et al., 2008), and scopolamine has been shown to be a potent rapid antidepressant (Furey and Drevets, 2006).

2.3. Selective breeding for differential rates of 50 kHz and 22 kHz USVs

In order to further elucidate the molecular mechanisms that are involved in the regulation of positive and negative emotional states, rats were selectively bred for differential rates of hedonic 50 kHz USVs (Burgdorf et al., 2005, 2008). Animals selectively bred for low rates of 50 kHz USVs (Low Line) had a concomitant increase in 22 kHz USVs and showed elevated levels of anxiety in the open field, in the social contact test, and in infant distress vocalization tests

compared to randomly bred animals (Burgdorf et al., 2008). Conversely, animals selectively bred for high rates of 50 kHz USVs (High Line) had a concomitant decrease in the 22 kHz USVs, showed lower levels of anxiety in the open field test, decreased rates of aggression, and increased sensitivity to sucrose reward compared to randomly bred animals (Burgdorf et al., 2008). These animals have been selectively bred for 18 generations to date and have displayed stable differences in USVs from adolescence through adulthood (3 months).

Studies on the molecular mechanisms associated with the USV patterns of the High Line and Low Line animals to date are consistent with depression-resilient and depression-prone phenotypes as discussed above. For example, High Line animals exhibited higher levels of the μ -opiate acting Met-enkephalin-like immunoreactivity in the hypothalamus and ventral tegmental area and other related limbic structures (Burgdorf et al., 2008). Injections of the μ -opiate agonist DAMGO into the ventral tegmental area increased rates of 50 kHz USVs and was rewarding to the animals (Burgdorf et al., 2007). Low Line animals exhibited higher levels of cholecystokinin-like immunoreactivity in the posterior neocortex. Cholecystokinin (CCK) content in the posterior cortex was elevated by social defeat, and was correlated with 22 kHz USVs rate of the defeated animal (Panksepp et al., 2004). It has also been shown that social defeat, which elevates levels of 22 kHz USVs, increased CCK-like immunoreactivity in cortical microdialysates (Becker et al., 2001) and CCK administration promoted social defeat-induced behaviors including 22 kHz USVs (Becker et al., 2007).

3. Using hedonic USVs to uncover the novel molecular substrates of positive affect

Genes specific to positive affective states can be uncovered by examining transcripts that are upregulated by hedonic play, but not aversive social defeat (Burgdorf et al., 2010a). To this aim, we have developed an in-house fabricated focused microarray platform, which can detect families of genes that are specifically upregulated following hedonic rough-and-tumble play when coupled with appropriate bioinformatics tools. These mRNA changes are corroborated by quantitative qrtPCR and quantitative protein assays (Radioimmunoassay, ELISA, Western blots). These studies identified both the insulin like growth factor I (IGFI) and the NMDA NR2B receptor subunit as being specifically upregulated by hedonic rough-and-tumble play.

Function studies with IGFI and NR2B demonstrate that they play a regulatory role in positive affective states (Burgdorf et al., 2010a).

Intracerebroventricular (icv) injections of IGF1 increased hedonic USVs in an IGF1 receptor (IGF1R) dependent manner, whereas icv injections of an IGF1R specific small interfering RNA (siRNA) decrease rates of hedonic USVs. Peripheral injections of the NMDAR NR2B-preferring glycine site partial agonist, GLYX-13, increases rates of hedonic USVs, whereas the NR2B receptor antagonist, ifenprodil, decreases rates of hedonic USVs. Microinjections of GLYX-13 into the medial prefrontal cortex (but not dorsal control sites) increases rates of hedonic USVs (Fig. 1).

The modulation of glutamatergic transmission has become a major target in the development of antidepressants for biogenic amine anti-depressant resistant patients (Hashimoto, 2009; Machado-Vieira et al., 2009; Skolnick, 2009). NMDAR is a validated target for depression and GLYX-13 is entering phase II clinical trials for the treatment of depression. Recent human clinical studies with known NMDAR antagonists CP-101,606 and ketamine have found significant reductions in depression scores in patients with treatment-resistant depression. Ketamine was also shown to produce a robust antidepressant effect in patients with treatment-resistant bipolar disorder (Zarate et al., 2006). Although these drugs produced clinically unacceptable dissociative side effects, the efficacy in these studies was significant (>50% response rate in resistant subjects, fast onset of action, and long duration of effect up to 7 or more days following a single dose), and confirmed NMDAR as a novel target of high interest in the treatment of depression. Like ketamine, GLYX-13 produces a robust anti-depressant effect in the rat Porsolt test 20 min and 2 weeks post-injection (Burgdorf et al., 2010b). However, unlike ketamine or CP-101,606, GLYX-13 shows no sedative or dissociative side effects clinically or in pre-clinical models.

4. Conclusions

Affective neuroscience approaches to brain emotional systems provide convergent methodologies to decipher molecular mechanisms for the generation of a variety of positive affective states. We are confident that indices such as 50 kHz USVs express positive emotional states because all of the brain sites localized in SEEKING circuits that generate these sounds also sustain self-stimulation behavior (Burgdorf et al., 2007), a critical criterion for positive affect processes of the brain. The implications of these developments in psychiatric medicine are becoming more evident. For instance, depressive disorders can be ameliorated by promoting various positive emotions, whether psychobehaviorally or pharmacologically.

The discovery that 50 kHz ultrasonic vocalizations (50 kHz USVs) reflect a positive affective state in rats allows this measure to be used effectively to monitor hedonic states in animal models of addictions (Panksepp et al., 2002) as well as various pre-clinical models of psychiatric disorders characterized by imbalance mood states, especially depression. Our working hypothesis is that these vocalizations directly reflect bursting of ventral tegmental dopamine neurons within the mesolimbic reward-SEEKING dopamine circuits. Our prediction is that 50 kHz "chirps" are emitted in close relationship to the bursting of dopamine cells, a neurophysiological condition that promotes active dopamine release.

In addition, by studying diverse forms of positive pro-social emotional states – LUST, CARE, and PLAY – the neuroanatomical basis and molecular mechanisms of various types of positive affect can now be understood. These findings have direct implications for new therapeutics in biological psychiatry as well as psychotherapeutic approaches to achieve affective homeostasis. The use of affective neuroscience approaches has led to the discovery that the NR2B NMDA receptor subunit plays functional role in hedonic USVs. GLYX-13 (an NR2B preferring glycine site partial agonist) is in phase 2 clinical trials for the treatment of depression.

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