Preclinical Modeling of Primal Emotional Affects (SEEKING, PANIC and PLAY): Gateways to the Development of New Treatments for Depression

Jaak Panksepp\textsuperscript{a} Yoram Yovel\textsuperscript{b}

\textsuperscript{a}Department of Integrative Physiology and Neuroscience, College of Veterinary Medicine, Washington State University, Pullman, Wash., USA; \textsuperscript{b}Institute for the Study of Affective Neuroscience, University of Haifa, Haifa, Israel

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Abstract
Mammalian brains contain at least 7 primal emotional systems – SEEKING, RAGE, FEAR, LUST, CARE, PANIC and PLAY (capitalization reflects a proposed primary-process terminology, to minimize semantic confusions and mereological fallacies). These systems help organisms feel affectively balanced (e.g. euthymic) and unbalanced (e.g. depressive, irritable, manic), providing novel insights for understanding human psychopathologies. Three systems are especially important for understanding depression: The separation distress (PANIC) system mediates the psychic pain of separation distress (i.e. excessive sadness and grief), which can be counteracted by minimizing PANIC arousals (as with low-dose opioids). Depressive dysphoria also arises from reduced brain reward-seeking and related play urges (namely diminished enthusiasm (SEEKING) and joyful exuberance (PLAY) which promote sustained amotivational states). We describe how an understanding of these fundamental emotional circuits can promote the development of novel antidepressive therapeutics – (i) low-dose buprenorphine to counteract depression and suicidal ideation emanating from too much psychic pain (PANIC overarousal), (ii) direct stimulation of the SEEKING system to counteract amotivational dysphoria, and (iii) the discovery and initial clinical testing of social-joy-promoting molecules derived from the analysis of the PLAY system.

A premise of affective neuroscience \cite{1,2} is that studies of the primal emotional feelings of other animals – namely preclinical models where detailed neuroscientific inquiries may be conducted – can provide important basic information about imbalances in the underlying affective circuits that promote psychiatric disorders, with the possibility of developing more specific and effective psychiatric treatments. The affective neuroscience strategy takes animal emotional feelings seriously, yielding empirical findings about affective states that cannot be collected in humans. Of course, we recognize that emotional feelings in animals is a concept that has yet to be universally embraced, with many prominent investigators still claiming that we cannot ever study emotionally valenced states of mind in other animals \cite{3} or that they simply may not exist \cite{4}. We be-
lieve such skeptical perspectives overlook critical information, such as the fact that we can evoke coherent emotional action tendencies with deep-brain stimulation (DBS) in homologous neural regions in all mammals that have been tested [1, 5, 6], and that such state shifts are demonstrably rewarding and punishing to animals, in the sense that animals ‘willingly’ turn them on or off, respectively, which is the decisive empirical evidence for affective states, namely valenced experiences, in non-speaking animals [7–9].

Credible arguments against such interpretations of objective data for emotion-related affective states in animals remain to be provided by those who assert that affective states cannot be scientifically studied in animal models [3, 4]. There are only stipulative claims that ‘We will never know what an animal feels’ [3, p. 666] (our emphasis). We think this mindset, reaffirming classical behaviorist dogma, overlooks abundant evidence, namely that artificial (e.g. DBS) arousal of brain emotional circuits in animals provides objective measures of positively (rewarding) and negatively (punishing) valenced feelings [1, 2]. Such replicable findings support our evidence-based conclusion that all other mammals do experience their emotional arousals, and that a more detailed study of such brain systems may provide unique opportunities for decoding which emotions, and which of their regulatory neurochemistries, may be involved in particular psychiatric disorders [10, 11], hopefully providing novel ideas for clinical therapeutics. Here we discuss how such a strategy is currently impacting our search for evidence-based new treatments for depression [for a more extensive discussion of relevant details, see 12].

This only seems like a radical new approach because of an outdated behaviorist tradition (of ultimate skepticism with the possibility that science can ever illuminate internally experienced psychological states in animals), which still governs much of animal behavior research, where discussions of the internal feelings of other animals was consistently discouraged during the 20th century. We think this bias is outdated since it is counteracted by concrete evidence for 7 primary emotional processes – SEEKING, RAGE, FEAR, LUST, CARE, PANIC/GRIEF and PLAY (capitalization reflects a proposed primary-process terminology, to minimize semantic confusions and mereological fallacies), namely neural circuits that evolution built into mammalian brains, with affect indexed by the capacity of localized subcortical DBS to evoke coherent emotional action tendencies, which by objective measures of reward and punishment are interpreted to have affective contents. For instance, animals allowed to make free ‘choices’ eagerly turn on DBS, within what we call the SEEKING, LUST, CARE and PLAY systems; they turn off DBS that evokes RAGE, FEAR and PANIC (namely social distress) types of behavioral responses. These effects are obtained by DBS applied only to subcortical brain systems, where homologous evolutionary brain organization patterns abound, namely midline thalamic and mesencephalic, and most especially hypothalamic, brain regions [1, 5, 6]. Importantly, abundant validation is available for comparable DBS manipulations in humans [13, 14], who can report their affective experiences verbally in real time as well as reflectively. Perhaps the most striking example in the literature is of a patient who received DBS to the periaqueductal gray, where various primal emotional systems converge, especially FEAR: a dramatic affective shift was observed, with the patient exclaiming suddenly that he was ‘scared to death’ [14]. There are many comparable reports for other emotional states, evoked in humans by DBS to the same brain regions from which DBS in animals leads to the distinct corresponding emotional behaviors [for a review, see 13].

Since such positively and negatively valenced effects (arising from evolutionarily related emotional network arousals) generalize across species, we would suggest that DBS studies in animals provide a much underutilized way to understand specific emotional systems that control human moods relevant for understanding and treatment of human psychopathology. Again, since this point seems not to have received adequate attention in the preclinical modeling of psychiatric disorders, most of which remains resolutely behavioristic, we emphasize that empirical evidence for affective changes in animals is provided by the rewarding and punishing properties of the above types of DBS. This interpretation is congruent with the fact that humans have neither rewards nor punishments that they do not experience in positive or negative valenced/affective ways. We thus work from the premise that affective phenomenal forms of consciousness (valenced emotional, homeostatic and sensory qualia) may have been the first forms of experience to have emerged in brain evolution. We do not mean that these valence effects necessarily imply ‘awareness’, which is an intrinsically ambiguous word in consciousness studies, suggesting ‘knowledge of one’s experiences’. Our position is simply that other animals do have raw phenomenal experiences, namely affective qualia, which is advanced with the related proposal that the neural circuits that produce such valenced experiences are also critical for learning (e.g. the mechanisms of ‘reinforcement’) [2].

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Evolutionary Levels of Control in Mammalian Brains

In pursuing such issues, we focus on the evolutionary ‘layering’ that is evident in brain organization which, for didactic convenience, can be divided into ‘primary’ (e.g. deeply subcortical unconditional/instinctual emotional response systems), ‘secondary’ (intermediary learning and memory mediated by the basal ganglia) and ‘tertiary’ (neocortical) cognitively rich brain processes that may mediate higher-order syntactic thoughts in Edmund Rolls’s [4] intriguing hypothesis. Obviously, the lower affective brain functions (which deserve a distinct nomenclature) are more capable of being empirically studied than any higher-order syntactic thoughts, which currently remain empirically impenetrable in nonspeaking animals; see figure 1 for the kinds of terminologies that are widely used in current discussions of the traditionally labeled ‘brain reward system’, discovered over 60 years ago. Most contemporary investigators agree that this system does not mediate feelings of sensory-type pleasures, but instead, something very different; we think a proper affective descriptor for this evoked state is exuberance, for that is the state that accompanies enthusiastic foraging/exploration in humans, and those are the explicit behaviors evoked by DBS of the medial forebrain bundle (MFB), the neural substrate for SEEKING, which has only recently been described in humans with modern in vivo imaging tools (see Coenen et al. [15], with extensive neuroanatomical descriptions) that have suggested immediate clinical implications [16, 17].

In sum, it is much easier to study the valenced evolutionarily constructed subcortical experiential functions in animals than to study developmentally constructed higher brain functions (e.g. cognitive higher-order syntactic thoughts). Further, we postulate that it is currently more important to fathom the lower affective functions of mammalian brains for generating treatment-relevant understandings of human psychopathologies than are attempts to understand higher brain functions in animals, since their ‘thoughts’, if they exist, are surely nonverbal (perhaps visual) and currently empirically impenetrable. We admit that our conceptual position may be only an empirical/ontological approximation, especially when contrasted to affective qualia in humans (e.g. the qualitative feel of emotions in different animals may be as different as their homologous body structures, most especially when raw feelings are blended with cognitions), but it is at least a workable empirical approach that remains much underutilized. Here we advance the view that such affective modeling can yield new medical treatments more rapidly than simply focusing on behavioral processes in animals. In sum, we propose that the neglect of affect in preclinical psychiatric modeling may be a major reason why no truly new psychiatric medicinal treatments have arisen from behavior-only preclinical modeling so far [18]. To support this view, we will describe 3 new experimental treatments for depression that we have helped de-
velop and implement, based on cross-species affective neuroscience thinking – a strategy that claims to have provided a preliminary characterization of the primal emotional underpinnings of human depressive disorders by modeling relevant affective processes in animals, namely the 3 described below.

Three Key Emotional Systems Implicated in Depression

Here we focus on only 3 primal emotional systems – namely SEEKING, PANIC and PLAY – that we propose are critically important for understanding depression-related affective changes. As such, they may presumably be modulated by new medications and perhaps old ones, like opioids (e.g. buprenorphine), used at very low/safe doses. Throughout this discussion, because of space limitations, we will not discuss the role of classical biogenic amine systems, such as norepinephrine or serotonin imbalances, in depression; biogenic amines modulate multiple (indeed all) affective functions, including emotional ones. Indeed, they were the first to be implicated in the genesis of depression (for a detailed summary of such data, see Watt and Panksepp [19]).

First Hypothesis: Overactivity of the PANIC System Might Promote Depressive Feelings by Promoting ‘Psychological Pain’

Ever since the theoretical work of John Bowlby [20], it has been known that excessive early separation experiences, and poor parental bonding, increase the susceptibility to future depression. Using this logic, and knowing how powerful opioids are in reducing separation distress in all species ever tested [1, 9, 21], we postulated that pharmaceuticals that are very effective in reducing separation distress (i.e. PANIC arousal) might be novel antidepressants.

Recently investigators are increasingly entertaining the possibility of using such ‘safe opioids’ for the treatment of depression, as well as the chronic ‘psychological pain’ that often promotes suicidal ideation [22]. To be a ‘safe opioid’, the analgesic effects and the lethal (respiratory depression) effects of a particular opioid ligand need to be dissociated. Buprenorphine, a partial agonist at μ-opioid receptors (i.e. stimulating opioid receptors at low doses, but blocking them at high doses), is just such a drug [23, 24]. Indeed, across the past 30 years there have been sporadic open studies demonstrating that very low doses of buprenorphine can have robust and rapid antidepressant effects [25, 26].

Accordingly, we designed a double-blind, placebo-controlled pilot study of the effects of low-dose buprenorphine on depressed, nonpsychotic individuals with sustained thoughts about committing suicide. The study assessed their depressive and suicidal symptomatology at weekly intervals with scales noted in figure 2. Namely, the severity of depressive symptoms and suicidality was measured with the respective Beck scales at 3 time points: immediately before, 1 week after and 2 weeks after the onset of sublingual buprenorphine or placebo treatment (initial dose = 0.2 mg/day, gradually increased to 1.6 mg/day in divided doses). The study drug was given as an adjunct to the treatment that the study participants were already receiving upon their recruitment to the trial. This preliminary feasibility study indicated that 2 weeks after the onset of treatment, buprenorphine was distinctly more effective than placebo in reducing depressive symptoms as well as suicidal urges and ideation (fig. 2).

Despite such encouraging findings, this pilot study suffered from several limitations. First, by design, the study population consisted of depressed and personality-disordered outpatients who were recruited into the study shortly after they had made a suicide attempt/gesture, namely at a time of crisis in their lives. This resulted in high noncompliance and dropout rates that reduced the number of our pilot study completers (n = 4 and n = 6 for the buprenorphine and placebo groups, respectively). Second, the small number of study completers, as well as the fact that for ethical reasons all study participants continued to receive ongoing treatments, including antidepressant medications at the discretion of their treating clinicians, resulted in significant demographic and baseline clinical differences between the treatment and placebo groups, which in addition took the study medication (buprenorphine or placebo) in a double-blind design. The small size and the heterogeneous nature of the study population precluded any assessment of the statistical significance of these findings. Third, the placebo-controlled effects of buprenorphine on depression and suicidality were only apparent after 2 weeks of treatment, but it was unclear whether they might diminish or increase further with time. Still, since the treatments (buprenorphine or placebo) were administered in a double-blind fashion, we decided to proceed to a more extensive study.

In light of the encouraging findings as well as the limitations of the pilot study, a larger, 4-week, multicenter, double-blind, placebo-controlled phase 3 clinical trial of
buprenorphine as an adjunctive treatment for depressive and suicidal psychopathology was designed; recruitment is currently completed (with \( n = 40 \) and \( n = 20 \) study completers in the buprenorphine and placebo groups, respectively).

**Second Hypothesis: Reduced SEEKING Activity Can Diminish Enthusiasm for Life**

The ‘brain reward system’ concept has been with us since 1954, ever since James Olds and Peter Milner [27] found self-stimulation behavior for self-administered DBS in animals. The most robust effects were obtained from the MFB that courses upward from the ventral tegmental area situated at the base of the brain at the midbrain-hypothalamic transition, distributing neural influences, especially dopamine release, to various terminal regions such as the nucleus accumbens and further up medial frontal cortical regions, long implicated in emotional processing [for anatomical summaries, see 1, 15]. The evoked feeling from MFB-DBS has been presumed to be in a vaguely defined ‘pleasure’ category, but the possibility that the feeling resembles any sensory pleasure known to man seems remote. Based on ethological changes during relevant animal DBS studies, namely induction of intense exploration and foraging, accompanied by forward locomotion and sniffing, we have called this massive, affectively positive ‘brain reward’ emotion circuitry the SEEKING system to better convey the affect of ‘enthusiasm-exuberance’ that this system probably mediates [1, 2].

There has been abundant evidence for the obvious hypothesis that this system mediates affective feelings organisms have when they energetically engage their environments in the search for all kinds of resources [1, 2, 5, 6]. Still, the old ‘brain reward’ nomenclature is still most commonly used, although newer concepts have been emerging for a long time [see fig. 1, for 3 major proposals, which may be good descriptors of primary (SEEKING), secondary-learning (‘wanting’) and tertiary-cognitive (‘reward prediction error’) levels of neuronal processing]. It has also long been known that damage to this system produces a global depressive type of behavioral change, where animals stop exploring their environments and stop self-grooming. They often stop eating and drinking, and may die if not carefully nursed back toward self-maintenance. However, the traditional ‘pleasure/reward

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**Fig. 2.** Results of a small double-blind, placebo-controlled pilot study that assessed the severity of depressive symptomatology and suicidal urges of depressed, acutely suicidal, nonpsychotic individuals at 3 time points: immediately before, 1 week after and 2 weeks after the onset of sublingual buprenorphine or placebo treatment (initial dose = 0.2 mg/day, gradually increased to 1.6 mg/day in divided doses). a Two weeks after the onset of treatment, buprenorphine was more effective than placebo in reducing depressive symptoms as measured by the Beck Depression Inventory (BDI)-II [79]. b Two weeks after the onset of treatment, buprenorphine also appeared to be more effective than placebo in reducing suicidal urges and ideation, as measured by the Beck Scale for Suicide Ideation (BSI) [80]. However, the small number of study completers (\( n = 4 \) and \( n = 6 \) for the buprenorphine and placebo groups, respectively) and the heterogeneous nature of the study population do not allow assessment of the statistical significance of these findings. A larger 4-week study has been under way.
system’ interpretation for MFB arousal [28] – which a few others, beside ourselves [1, 2, 5, 6, 29], are beginning to critique as basically incorrect, especially after the ‘paradoxic’ findings of Berridge and Valenstein [30] – still remains the commonly preferred historical label (for a full discussion of various current schools of thought, see Panksepp and Moskal [31]). In any event, the ethological behavior patterns provoked by DBS of this circuitry do not closely resemble the ‘consummatory reward’ phase of appetitive behavior, but rather the natural exploratory-foraging phases of motivated behavior that is so ubiquitous before any consummation can take place. Thus, the accompanying state of mind is more likely to be akin to intense ‘interest’ or ‘enthusiasm’, with ‘exuberance’, even ‘mania’, being reasonable descriptors of intense (even at time pathological) arousal – namely the spectrum of feelings that are depleted in depressed individuals. In short, this system promotes a specific type of psychobehavioral ‘arousal’ (another excessively general term), allowing organisms to energetically pursue all external rewards needed for survival and reproduction, with an accompanying psychological state that is unlikely to reflect consummatory ‘rewards’ (e.g. taste).

Our guiding hypothesis has been that excessive and prolonged social loss, as reflected in affectively negative overarousal of the PANIC system, would diminish SEEKING system activity [11, 19], an effect that we preclinically modeled with considerable success [29]. Accordingly, it was proposed that DBS of the MFB might be a robust antidepressant in treatment-resistant patients [15, 16]. Since neither the MFB-based SEEKING system nor the separation-distress-evoking PANIC system had ever been adequately characterized in humans, diffusion tensor imaging studies were conducted: as summarized in Coenen et al. [15], putative trajectories of these systems were readily defined, providing coordinates for potential MFB-DBS studies in depressed humans. In other words, DBS-induced affective shifts might restore ‘enthusiasm’ in depressed patients, and help them to again engage positively with the world, an interpretation that could be predicted from abundant preclinical work [1, 2, 5, 6, 31].

Results from the first 7 patients undergoing DBS at the University of Bonn medical center in Germany were published about a year ago [17]. The positive effects in 6 of the 7 patients were spectacular, with an antidepressant effect in treatment-resistant individuals evident within the first day of treatment. These benefits were sustained for as long as the patients have been followed so far (well over a year), and psychiatric benefits were more rapid and robust than alternative sites that have been studied, including the nucleus accumbens, which Schlaepfer et al. [32] pioneered as a target for DBS treatment of depression. Our interpretation is that facilitation of the SEEKING/enthusiasm urge is able to counteract the psychological pain engendered by sustained overactivity of the PANIC/separation distress system [19, 29, 31, 33].

Third Hypothesis: Depression May Often Reflect Chronically Diminished Capacity for Social Joy

As our final example of the affective neuroscience strategy for the identification of potential new antidepressant therapies, congruent with the previous hypotheses, we postulated that increased PANIC, along with diminished SEEKING and PLAY arousability, produces a dysphoric state in which individuals have a reduced capacity to experience social joy. Thus, we wondered whether we might be able to model such a potential antidepressive strategy in laboratory rats. Since we developed standardized procedures that anyone can use to study rough-and-tumble PLAY in laboratory rodents [34], we eventually discovered vocal indicators of social play, akin to the laughter of joy, in laboratory rats [35], which were especially useful for addressing such affective questions.

Although by traditional behavioristic standards this may sound like an improbable discovery, it is currently generally agreed that rats have a fairly simple repertoire of emotional vocalizations, mostly in the ultrasonic range (inaudible/undetectable to human hearing without specialized equipment) that reflect positive and negative emotions [36, 37]. Positive ultrasonic vocalizations (USVs) in the 50-kHz (+ or – 20 kHz) range can be promoted by a variety of positively valenced social activities – from the seeking of social or sexual companionship to predictable joys such as daily feeding periods in hungry rats, or the receipt of rewarding brain stimulation or being tickled [38, 39]. As already noted, USVs that occur around 50 kHz are emitted at particularly high rates during social play [40], preceding copulation [41] and in anticipation of or following the administration of cocaine or amphetamine [42, 43]. Currently, abundant evidence supports approximately 50-kHz vocalizations being used as indicators of appetitive positive affect, specifically of what we deem increased appetitive SEEKING system activity [44] – the fundamental substrate for appetitive enthusiasm (which has inadequately been conceptualized as ‘brain reward system’ activity) – appears to be underactive in most animal models of depression [19, 29, 45]. In contrast, the 22-kHz ultrasonic ‘complaints’ of rats ap-
pears to be a general indicator of negative mood states including PANIC (separation distress) alarm [29].

That 50-kHz chirps represent positive affect is dramatically supported by the finding that animals will self-stimulate sites in the brain shown to reliably increase 50-kHz vocalizations [44], and that these calls are abolished with all aversive stimuli while being increased by cues that predict a variety of rewards [for overviews, see 37, 39]. Evidence that 50-kHz calls represent a general appetitive state includes experiments demonstrating that such calls are emitted in anticipation of all positive rewards, from play to rewarding brain stimulation and drugs of abuse [37, 43, 44, 46]. In addition, appetitive approach behaviors have been recorded in response to playback of 50-kHz calls [47, 48]. Further, tickling animals, one of the best behavioral ways to evoke 50-kHz calls under full experimental control [37, 39], can robustly increase neurogenesis in the hippocampus, a well-established neural effect of antidepressants [49].

The power of 50-kHz vocalizations as a measure of positive affect comes most dramatically from knowledge about the underlying neural system necessary for their production: lesions to either the dopamine-rich neuronal fields of the ventral tegmental area or the MFB (through which all monoamine systems, namely dopamine, norepinephrine and serotonin, ascend from their cell bodies that are located lower in the brainstem) produce an amotivational syndrome resembling severe depression. Such lesions also dramatically reduce 50-kHz USVs, particularly the frequency-modulated type chirps, which are the best indicators of positive affect [44].

All positive social situations reliably elevate positive-affect indicative chirps. Likewise, manual human tickling reliably evokes robust chirping calls in young rats and can be continued into adulthood with stable response rates [35, 49, 50], especially in animals that had received abundant tickling during adolescence. This tickling procedure, systematically used (fig. 3), has provided the first ‘psychoassay’ for positive social affect that is easy to implement using short 2-min assays, in which 15-second no-tickling periods are followed by 15-second tickling periods [37, 39].

Indeed, natural play has now been effectively used to screen for brain factors that may increase appetitive positive affect, as a way to guide discovery of brain neurochemical systems that may yield antidepressant effects. Panels of brain chemical candidates were derived using genetic microarray technologies by screening which molecular pathways were significantly modulated by consistently happy social play. Oligonucleotide microarray in-dices of gene expression profiles were harvested from the neocortices of animals that had experienced strictly positive play (no emission of 22-kHz USV complaints). Expression profiles for 1,200 genes were monitored 1 and 6 h after the end of such play sessions; about one third of the genes exhibited significantly changed expression patterns in frontal executive-motor and posterior sensory-perceptual projection regions of the neocortex [51, 52]. Indeed, we have found that infusions of insulin-like growth factor-I (one of the most intensely expressed genes) into the ventricular system of rats elevated 50-kHz chirping, whereas chemical blockade of the insulin-like growth factor-I receptor system reduced this index of positive affect [53].

A second candidate antidepressive neurochemical pathway (based on strong gene expression) was a receptor for the NR2B subunit of the glutamate-glycine receptor complex. Through a series of experiments at the Falk Center for Molecular Therapeutics described elsewhere [51, 52], potential medicinal agents were constructed that might interact in therapeutical ways with glycine receptors, in both facilitatory (agonist) ways at low doses and inhibitory (antagonist) ways at higher doses. A potentially useful medicinal vector was identified, code-named GLYX-13 (a tetrapeptide with the sequence threonine-proline-proline-threonine), which is a partial agonist for glycine sites on N-methyl-D-aspartate receptors, promoting glutamate transmission in low doses and blocking it in high doses. Fortunately, this positive social affect-facilitating medicinal agent sailed smoothly through animal and human toxicology trials and is currently in the FDA-approved phase 2B of clinical testing, passing phase 2A with effect sizes considerably larger than those of selective serotonin reuptake inhibitors [Moskal et al., unpubl. data]. GLYX-13 represents a totally novel antidepressant concept. If it proceeds through the whole gamut of FDA testing, namely the remaining phase 3 double-blind, placebo-controlled evaluation, it may be the first novel antidepressant that has arisen from preclinical research. Again, the key affective neuroscience finding was that PLAY elevates relevant gene expressions in the brain, and that a resulting medicinal agent, GLYX-13, promotes positive affective 50-kHz USVs in rats during social play, which can be simulated by tickling rats and thereby indirectly activating the SEEKING system [39, 51].

In fact, our novel tickling technique (easily learned) is the first rigorous preclinical ‘psychoassay’ (fig. 3) for positive social feelings that may be effectively used to identify not only depressogenic states in animals, but also new antidepressant factors. Further, we use the assay routine-
ly to analyze individual phenotypic variability in outbred rats for their resistance to depression. It is known that the level of responsivity to tickling is a stable affective phenotype with animals exhibiting high levels of chirps generally being resistant to depressogenic stressors, and animals that exhibit low levels of chirping tending to be depression prone. Indeed, these phenotypic differences can be easily bred. Parenthetically, another method that we are actively pursuing is to create a simple assay to measure ‘spontaneous’ 50-kHz calls when animals are placed into a neutral, nonstimulating test environment. Such tests may be especially sensitive to temperamental differences in positive affect, without any imposed stimulation.

On the negative side of the affective spectrum, there are reasons to believe investigators could use 22-kHz USVs in rats to index negative affect that may be relevant for understanding depression. Unlike 50-kHz calls, these vocalizations are related to affectively negative brain regions, such as the dorsal periaqueductal gray, where stimulation produces strong aversive states accompanied often by 22-kHz USVs. Indeed, animals that are simply allowed to listen to playbacks of 22-kHz USVs show neural activity in the periaqueductal gray. Another simple assay that may be implemented to evaluate sensitivity to this kind of negative affect is the use of repeated air puffs to the nape of the neck. Recently, one group of investigators used this method successfully to analyze affective changes resulting from heroin or alcohol withdrawal; they found that besides increased immobility in the forced swim test, withdrawal from either heroin or ethanol led to high rates of 22-kHz vocalizations, whereas controls produced hardly any such calls.

**Contextual Issues**

Because of space limitation, we have not discussed the many other animal/preclinical models in this vast field of research. They include various inescapable shock/stress procedures, often with supposedly unfelt ‘fear’ measures such as unconditional freezing (as well as associated changes in fear-related memories), swimming test (Porsolt test), sucrose intake as an index of anhedonia, and many others. Perhaps the most robust brain-manipulation-based model of depression is olfactory bulbectomy in rodents. We note that detailed coverage of most of these other models, widely used, is available in a special issue of *Neuroscience and Biobehavioral Reviews* edited by Markou. Of course, we also use behavioral, especially ethological, techniques to study depression, but what is new in our approach is a direct focus on specific affective...
circuits to evoke depression as well as to monitor the depressive cascade. This distinguishing feature has perhaps allowed us more success in novel treatment development than has been typical in the field.

In our coverage here we have not focused on traditional therapies, many of which also modulate the brain reward SEEKING circuitry, which is heavily influenced by all the biogenic amines – e.g. dopamine, norepinephrine and serotonin [for a full summary, see 19], as also highlighted by antidepressant dopamine agonists and reuptake inhibitors such as bupropion [62]. Further the discovery of a glycine receptor partial agonist (GLYX-13), currently in human testing, converges on the exciting work on brain changes in glutamatergic transmission that may underlie depression: As is well known, there is currently intense interest in drugs like ketamine to modulate N-methyl-D-aspartate transmission, which can yield rapid antidepressant effects [63]. Indeed, in line with findings with GLYX-13, an additional exciting breakthrough is the ability of glycine partial agonists such a D-cycloserine to strengthen psychotherapeutic benefits through reconsolidation of problematic memories with less negative affect, perhaps by amplifying the therapeutic effects of positive affects during therapy (for a full coverage, with extensive commentaries, see the forthcoming discussion with Lane et al. [64]). Of course, there is also an extensive focus on brain neuropeptide imbalances that contribute to depression, with exciting possibilities in the reduction of stress-induced negative affect with corticotropin-releasing factor antagonists [65, 66].

Further, we have not considered the common comorbidities, such as anxiety, that often accompany depression. This has long been widely recognized, and the use of benzodiazepine antianxiety agents is often highly beneficial during the first weeks of antidepressant treatment [67, 68]. For a fuller discussion of many of these issues, especially the vast neurochemical complexities, see Watt and Panksepp [19]. However, as emphasized in that review, as well as elsewhere [69, 70], we believe that a key issue that has been neglected are the actual evolved affective/emotional systems in all mammalian brains that are the ultimate mediators of affective states in humans. Neurosciences has done a superb job of revealing the vast neurochemistries and brain anatomies that contribute to depression, while concurrently largely neglecting the primary-process affective systems of mammalian brains as targets of inquiry. We feel the time is ripe to make a readjustment in priorities: by actually focusing on the nature of the diverse negative and positive affects of the brain, perhaps we will make more rapid progress in develop-
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