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Ultrasonic Vocalizations as Indices of Affective States in Rats

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Adult rats spontaneously vocalize in ultrasonic frequencies. Although these ultrasonic vocalizations (USVs) have been described as by-products of locomotor activity or social signals, accumulating evidence suggests that they may also index anticipatory affective states. Converging ethological, pharmacological, and brain stimulation research indicates that whereas long low-frequency (> 0.3-s, ~22-kHz) USVs occur during anticipation of punishment or avoidance behavior, short, high-frequency (< 0.3-s, ~50-kHz) USVs typically occur during anticipation of reward or approach behavior. Thus, long 22-kHz USVs may index a state of *negative activation*, whereas short, 50-kHz USVs may instead index a state of *positive activation*. This hypothesis has theoretical implications for understanding the brain circuitry underlying mammalian affective states and clinical applicability for modeling hedonic properties of different psychotropic compounds.

Do rats have feelings, and if so, how would we know? In his essay "What Is It Like To Be a Bat?" philosopher Thomas Nagel concluded that humans can never know what it is like to be a bat (or, by extension, a rat), because humans lack the same sensory apparatus as bats (Nagel, 1974). For instance, bats use reflected sound and hearing to echolocate, whereas most humans use reflected light and vision to navigate. This sensory specialization is reflected in the relative overdevelopment of auditory cortex in bats compared with the relative overdevelopment of visual cortex in humans. Thus, a lack of similarity in the representational capacity of sensory cortices provides one indication that bats and humans do not share the same sensory qualia, or qualities of experience.

However, subcortically, bats, rats, humans, and other mammals show more abundant anatomical similarities than differences. Beginning with the observation that electrical stimulation of specific subcortical pathways can elicit motivated behavior in rats (J. Olds & Milner, 1954), and followed by similar observations in all other mammalian species studied (M. E. Olds & Fobes, 1981), a few investigators have argued that mammals share evolutionarily conserved brain circuits that facilitate specific kinds of emotional arousal and social interaction (MacLean, 1990; Panksepp, 1998). According to these theorists, the existence of subcortical structural and corresponding functional homologies suggests that even mammals with different sensory capacities may experience similar affective qualia.

Even if different mammalian species (e.g., humans and rats) share some similar core affective experiences, how could the two be compared? Ideally, representatives of each species would be able to accurately inform researchers about their emotional experiences; the researchers could verify their self-reports with other behavioral measures; and then researchers could compare those accounts across species in order to identify similarities and differences. Such an endeavor seems possible in the case of humans but has been deemed impossible in the case of rats and other mammals that lack linguistic capacities. However, rats and other mammals do communicate vocally, and recent technological developments that allow humans to enhance their hearing to match the ultrasonic sensory capabilities of rats may open up opportunities for one such line of inquiry in the animal species most commonly studied by experimental psychologists—*Rattus norvegicus*. To provide a framework for comparing the affective qualia of humans and rats, we first review how humans describe their feelings and the implications of those self-reports for constructing a generalizable model of affective experience.

Inferring Affect in Humans

The human tendency to verbally express one's feeling state has been harnessed and measured with the help of psychometric instruments, in which respondents rate how strongly they feel with respect to a series of adjectives on numbered or continuous scales. Factor analytic studies of covariance among these ratings have repeatedly revealed that self-reported mood can be characterized by a two-dimensional space bisected by the independent dimensions of valence (running from positive to negative) and arousal (running from high to low; Feldman-Barrett & Russell, 1999; Watson, Wiese, Vaidya, & Tellegen, 1999). Rotation of valence and arousal dimensions by 45° yields two dimensions of affective arousal, named *positive activation* (PA) and *negative activation*

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(NA; see Figure 1). Several psychometric studies have demonstrated that adjectives such as *active, excited,* and *elated* describe states of high PA, whereas *drowsy, sleepy,* and *sluggish* describe states of low PA. In a similar fashion, adjectives such as *fearful, hostile,* and *nervous* describe states of high NA, whereas *calm, placid,* and *relaxed* describe states of low NA (Watson et al., 1999). While this two-dimensional framework provides a richer description of affective space than a unidimensional hedonic continuum (Young, 1959), it can also serve as a superordinate set of dimensions that describes important qualities of discrete emotions such as anger, disgust, fear, happiness, and sadness (Tellegen, Watson, & Clark, 1999). The relative independence of PA and NA dimensions implies that, in general, one cannot predict how much PA a person is feeling based on his or her current level of NA, and vice versa.

Humans also express their feelings nonverbally, and investigators have used nonverbal behaviors in attempts to validate these self-report indices of affect. For instance, humans show potentiation of the startle response (an unconditioned avoidance response) while viewing pictures that induce NA, but dampened startle response while viewing pictures that induce PA (Lang, Bradley, & Cuthbert, 1990). Similarly, electromyographic activity of muscles implicated in facial expression such as the corrugator supercilii (brow) correlates with self-rated NA induced by unpleasant slides, whereas activity of the zygomatic major (upper cheek) correlates with self-rated PA induced by pleasant slides (Bradley, Cuthbert, & Lang, 1990). These unconditional behavioral measures are most likely to correlate with self-reported affect at high levels of self-reported intensity (Cuthbert, Bradley, & Lang, 1996; Rosenberg & Ekman, 1994). Thus, the strongest validation of self-reported affect in humans comes from demonstrations of covariation among psychometric and behavioral indices of affect including increases in sympathetic tone, unconditioned emotional responses, and emotional expression. Naturally occurring covariation among these indices supports the notion that affective states have survival value in promoting a state of sustained readiness that can facilitate behavioral approach to rewarding stimuli or avoidance of punishing stimuli (Dicks, Myers, & Kling, 1968; Young, 1959).

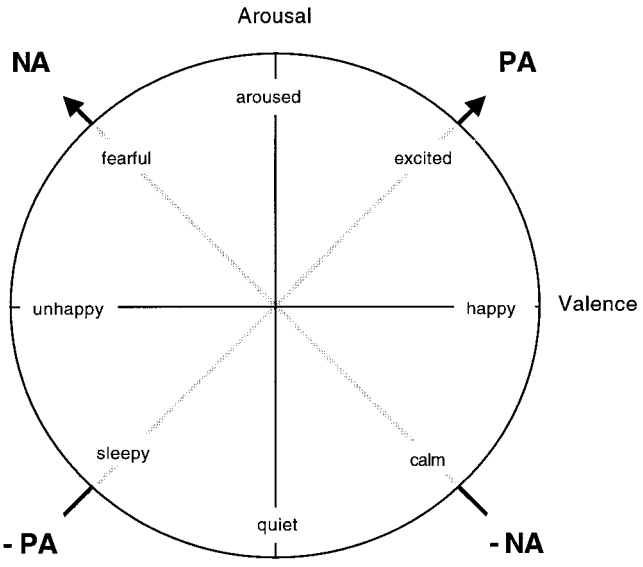

Figure 1. A two-dimensional map of affective space. Valence runs from negative (left) to positive (right), whereas arousal runs from high (top) to low (bottom). PA = positive activation; NA = negative activation. Adapted from "The Two General Activation Systems of Affect: Structural Findings, Evolutionary Considerations, and Psychobiological Evidence," by D. Watson, D. Wiese, J. Vaidya, and A. Tellegen, 1999, *Journal of Personality and Social Psychology, 76,* p. 821. Copyright 1999 by the American Psychological Association. Adapted with permission of the author.

Even with the help of self-report measures, inferring humans' affective reactions to various stimuli poses a methodological challenge. This dilemma is compounded in the case of nonhuman animals. With rodents, investigators have occasionally inferred the affective impact of various stimuli on the basis of conditioned behaviors (e.g., pressing a bar to obtain rewards or avoid punishments; J. Olds & Milner, 1954). However, unconditioned behaviors may also provide sensitive indices of animals' affective reactions. For instance, even without prior exposure or training, rodents typically approach and explore primary rewards (e.g., food, copulation) and avoid primary punishments (e.g., pain, predators; Panksepp, 1982, 1998). Most mammals also show other associated unconditioned or "instinctual" behaviors as well as autonomic arousal when initially exposed to stimuli that hold primary incentive value. For instance, rats increase their rate of sniffing when exposed to unconditional rewards (Rossi & Panksepp, 1992) but freeze and so stop sniffing in the presence of unconditional punishments. Rats also emit vocalizations (ultrasonic vocalizations; USVs) that occur above the range of human hearing (i.e., $>$ 20 kHz) in many different contexts that involve incentives (Sales & Pye, 1974). In this review, we explore the possibility that distinct types of USVs can index positive and negative affective states in adult rats.

Types of Rat USVs

Investigators have documented the incidence of different types of USV in several strains of laboratory and wild Norway rats (*R. norvegicus;* including Lister, Long–Evans, Sprague–Dawley, and Wistar strains; hereafter collectively referred to simply as *rats;* Sales & Pye, 1974). Three types of USVs can be clearly discriminated in sonograms on the basis of a combination of sound frequency and length criteria (Brudzynski, Bihari, Ociepa, & Fu, 1993; Miczek, Tornatzky, & Vivian, 1991). Different types of USV occur at different ages, with "40-kHz" USVs predominating during infancy (i.e., 4–16 days of age; Noirot, 1968), and "22-kHz" and "50-kHz" USVs occurring during adolescence and into adulthood and then waning in frequency with senescence (Panksepp, Knutson, & Pruitt, 1998).

Infant 40-kHz USVs have been most extensively documented in rat pups that have been separated from their mothers (Miczek et al., 1991; Sales & Pye, 1974); 40-kHz USVs range in length from 80–140 ms. Although these vocalizations have a mean sound frequency of 40 kHz, they tend to sweep frequencies ranging from 30–65 kHz in a U-shaped or inverted U-shaped pattern that grows increasingly clearer in shape from 10–16 days of age (Brudzynski, Kehoe, & Callahan, 1999).

Juvenile and adult rats emit a second, longer 22-kHz USV in several naturalistic contexts, including exposure to predators (R. J. Blanchard, Blanchard, Agullana, & Weiss, 1991), exposure to pain (e.g., evoked by footshock; Tonoue, Ashida, Makino, & Hata,

1986), defensive and submissive displays during intermale fighting (Thomas, Takahashi, & Barfield, 1983), and the refractory period following copulation (Barfield & Geyer, 1975a). In scenarios involving threat cues, emission of 22-kHz USVs has been behaviorally associated with tense, motionless crouching behaviors and strong expiratory movements (Brudzynski et al., 1993). Sonographically, 22-kHz USVs are typically both longer (ranging from 300–3,000 ms; Sales, 1979) and lower in sound frequency than 40-kHz USVs (ranging from 18–32 kHz with a typically narrow bandwidth of 1–6 kHz; Brudzynski et al., 1993). Compared with 40-kHz USVs, whose sonographic profile changes over the course of development (Brudzynski et al., 1999), the sonographic signature of 22-kHz USVs remains remarkably stable over many different eliciting circumstances (e.g., touch by an unfamiliar experimenter, electrical shock applied to the tail, infusion of cholinergic agonists into the anterior hypothalamus; Brudzynski, 2001). However, investigators have noted that 22-kHz USVs emitted by male rats during the refractory period following ejaculation may contain more pitch modulations than the comparatively monotonic 22-kHz USVs that occur in response to typical threat stimuli (e.g., defeat, tailshock; van der Poel & Miczek, 1991). However, because of the paucity of discriminant evidence at present, we treat these 22-kHz USVs as a single entity.

Juvenile and adult rats also emit a third, shorter and higher pitched 50-kHz USV in naturalistic contexts including juvenile solicitation of play (Knutson, Burgdorf, & Panksepp, 1998), male approach and ejaculation during copulation (McIntosh & Barfield, 1980), male and female social exploration (R. J. Blanchard, Yudko, Blanchard, & Taukulis, 1993; Brudzynski & Pniak, 2002), and male agonistic behaviors during fighting (Sales & Pye, 1974). Behaviorally, 50-kHz USV emissions have been associated with locomotor activity, rearing, and exploration (Fu & Brudzynski, 1994). The sound characteristics of 50-kHz USVs show little overlap with those of 22-kHz USVs, because they are both shorter in length (ranging from 20–80 ms; Fu & Brudzynski, 1994) and higher in sound frequency (ranging from 35–70 kHz with a bandwidth of 1–6 kHz; R. J. Blanchard et al., 1993). Like 22-kHz USVs, the sonographic signature of 50-kHz USVs remains nearly identical over a range of types of occurrences (e.g., those occurring spontaneously as well as those elicited by circumstances such as amphetamine (AMPH) injection and infusion of glutamate into the medial preoptic area of the hypothalamus; Wintink & Brudzynski, 2001). Existing sonographic data do not suggest further distinctions within this range. Because the functional significance of infant USVs has been extensively discussed elsewhere (Blumberg & Sokoloff, 2001; Panksepp, in press), and this review concerns adult rats, we focus exclusively on proposed functions of 22- and 50-kHz USVs.

Hypotheses of USV Function

Several hypotheses have been proposed regarding the possible functions of 22- and 50-kHz USVs in adult rats. These hypotheses fall into at least three distinct categories. Some accounts characterize USV emission as a mechanical by-product, others as an affective expression, and others as a social signal (Knutson, Burgdorf, & Panksepp, 1998). For instance, one mechanical by-product account posits that adult 22-kHz USVs are a vestigial remnant of infant 40-kHz USVs, which putatively developed as a part of an "abdominal compression reaction" mechanism that increases an infant's blood pressure and perfusion during periods of maternal isolation (Blumberg & Sokoloff, 2001). A second mechanical by-product hypothesis describes 50-kHz USVs as resulting from thoracic compression caused by forepaw impact during periods of vigorous locomotor activity (Blumberg, 1992).

Affective expression accounts postulate that USVs index affective states involving both high levels of arousal (Bell, 1974) and valenced expectations (Knutson, Burgdorf, & Panksepp, 1999). For instance, because 22-kHz USVs occur in different threatening situations, investigators have postulated that their occurrence indexes negative affective states akin to the anxiety invoked by anticipation of punishment (Brudzynski, 2001; Miczek et al., 1991; van der Poel & Miczek, 1991; Vivian & Miczek, 1993). Similarly, because 50-kHz USVs predominate in contexts involving potential reward, investigators have hypothesized that 50-kHz USVs index positive affective states akin to the eagerness or excitement invoked by anticipation of rewards (Knutson et al., 1999).

Finally, social signal hypotheses posit that USVs primarily enable or inhibit specific types of social interactions. For instance, playback of 22-kHz USVs induces freezing and avoidance behavior (Brudzynski & Chiu, 1995), whereas playback of 50-kHz USVs can facilitate copulation (Geyer, McIntosh, & Barfield, 1978). Although mechanical by-product, affective expression, and social signal accounts of USVs are not mutually exclusive, each generates different predictions about when USVs will occur and when they will not. Thus, the explanatory power of each of these different types of hypotheses can be empirically evaluated and compared. In this review, we first evaluate the explanatory power of the affective expression account and then address the ability of mechanical by-product and social signal accounts to provide alternative explanations for the incidence of USVs in adult rats.

If affective states promote the initiation and deployment of approach or avoidance behaviors, then they should occur in response to the presentation of stimuli that eventually evoke approach or avoidance. Specifically, indices of PA should coincide with and even predict the onset of approach behaviors, whereas indices of NA should coincide with and predict the onset of avoidance behaviors. Further, if these indices mark an affective state rather than simply the accompanying approach or avoidance behaviors, then incentive stimuli should evoke them even when effective behavioral approach or avoidance is not possible. Thus, rats should make more 50-kHz USVs when presented with reward cues, and more 22-kHz USVs when presented with punishment cues. Finally, emission of USVs upon stimulus presentation may also predict a rat's future tendency to approach or avoid that stimulus.

Evidence for Affective Hypotheses of USV Function

Below, we review studies that have focused on USVs in the context of four categories of stimuli that can powerfully elicit approach or avoidance behavior, progressing from stimuli that elicit relatively complex combinations of approach and avoidance to stimuli that predominantly evoke either approach or avoidance. These stimuli include both social and nonsocial incentives (e.g., electrical brain stimulation and pharmacological administration). Our goal is to evaluate whether presentation of stimuli that clearly

evoke approach and avoidance also differentially elicits 50- or 22-kHz USVs, respectively.

Social Incentive Stimuli

Mammals engage in several prototypical social interactions throughout the life span (Panksepp et al., 1998). Rats may actively seek out some types of interactions such as nurturance, play, social investigation, and mating but show a more ambivalent mix of approach and avoidance behaviors regarding other social interactions such as fighting (Taylor, 1976). However, any social encounter necessarily involves a complex and dynamically evolving exchange between two or more participants, including a combination of both approach and avoidance behavioral components (Panksepp, 1998; Siviy, 2000). In addition, most social interactions include at least two rats (unless they involve a rat and a human), and so it is often difficult in experimental research to attribute USVs to one subject or the other—such discriminations require anesthetizing or devocalizing one of the subjects. Finally, 22-kHz and 50-kHz USVs are not always simultaneously measured in experiments involving social interactions. Despite the complexity these issues confer to an analysis of the relation between social approach or avoidance behaviors and USV production, ethological studies offer one of the richest available repositories of information pertaining to USVs and so deserve extensive and primary consideration.

During rough-and-tumble play, juvenile rats pounce on the back of a partner with their forepaws while directing play attacks or "dorsal contacts" at the nape of their partner's neck. The partner may also rotate to a supine position to defend its neck against these play attacks, allowing the attacker to momentarily "pin" the defender to the ground (Panksepp, Siviy, & Normansell, 1984; Pellis, Field, Smith, & Pellis, 1997; Vanderschuren, Niesnik, & Van Ree, 1997). Because they require an initial approach, dorsal contacts represent the clearest appetitive component of the play-related behavioral repertoire (Siviy & Panksepp, 1987). Most juvenile rats readily approach opportunities to play and prefer to inhabit places where they have played before (Calcagnetti & Schechter, 1992; Normansell & Panksepp, 1990). In general, play elicits 50-kHz USVs, but not 22-kHz USVs. Individual rats also emit more 50-kHz USVs in places where they have previously played relative to equally familiar places where they have not played before. Play-experienced (but not inexperienced) rats also emit high rates of 50-kHz USVs when presented with a potential play partner across a screen that precludes immediate physical interaction. Of interest, when play occurs over several consecutive days, 50-kHz USVs on any given day predict dorsal contacts on the next day in a playing pair, but not vice versa (Knutson, Burgdorf, & Panksepp, 1998). Thus, 50-kHz USVs, but not 22-kHz USVs, not only co-occur with but also selectively predict the appetitive behavioral component of play in juvenile rats.

Familiar humans can emulate rough-and-tumble play by "tickling" juvenile rats or manually stimulating the back of the neck repeatedly and gently. As with conspecific play, juvenile rats readily approach opportunities for this type of interaction as well as associated cues. Accordingly, tickling and presentation of cues associated with tickling powerfully elicit 50-kHz USVs in individual rats (Panksepp & Burgdorf, 1999). Emission of 50-kHz USVs during tickling predicts the speed with which individual rats will subsequently run to the tickling hand (see Figure 2; Panksepp & Burgdorf, 2000). On the other hand, dorsal contact of adult rats by unfamiliar humans, which elicits avoidance behavior and freezing, primarily evokes 22-kHz USVs rather than 50-kHz USVs in individual rats (Brudzynski & Ociepa, 1992).

Adult rats do not exhibit the same degree of play behavior as juveniles. However, adult rats do emit abundant 50-kHz USVs when they repeatedly come in contact with familiar conspecifics under conditions that do not involve fighting. In one study, presentation of an anesthetized (and thus silent) conspecific evoked primarily 50-kHz USVs in individual adult rats during social investigation, with females emitting more of these vocalizations than males (R. J. Blanchard et al., 1993).

Predators and associated stimuli also evoke avoidance behaviors including freezing and flight in adult rats. In natural colonies, rats do not vocalize in the immediate presence of predators but do emit 22-kHz USVs for up to 30 min following contact with a predator while hiding in their burrows. The presence of conspecifics can potentiate these vocalizations (R. J. Blanchard et al., 1991). Rats also freeze when exposed to stimuli associated with predators, such as cat hair (Catarelli & Chanel, 1979). The presence of cat odor also inhibits emission of both playfulness and 50-kHz USVs (Panksepp, 1998; Panksepp & Burgdorf, 1999), although the unconditioned effect of cat hair on emission of 22-kHz USVs remains to be examined.

Experienced male rats prefer places and smells associated with prior sexual interactions, and prior experience of mounting alone without intromission or ejaculation is sufficient to evoke these preferences (Stern, 1970). Female rats also prefer places associated with prior sexual interactions, provided the female had paced the timing of those interactions (Paredes & Alonso, 1997). Adult rats emit both 50- and 22-kHz USVs during mating, but these vocalizations coincide with different components of the mating behavioral repertoire. Prior to copulation, rats exhibit a species-typical

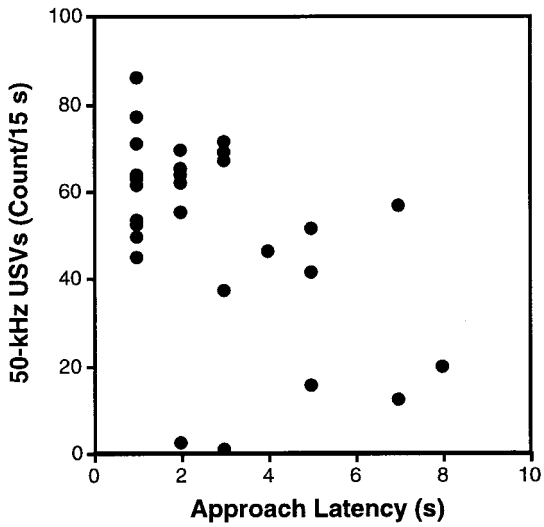

Figure 2. Average tickle-induced 50-kHz ultrasonic vocalizations (USVs) of five 15-s sessions predict subsequent latency to run to the tickling hand in isolate-housed juvenile rats ($n = 29$; $r = -.50$, $p < .005$); taken from Day 4 of Experiment 3 in Panksepp and Burgdorf (2000).

pattern of courting behavior, including pursuit, grasping, and mounting on the part of the male and proceptive behaviors such as hopping, darting, and ear wiggling on the part of the female. Both males and females emit 50-kHz USVs during this appetitive period (Sales, 1972). Males emit bursts of 50-kHz USVs during appetitive behaviors such as mounting and intromission and in synchrony with the consummatory response of ejaculation (McIntosh & Barfield, 1980; McIntosh, Barfield, & Thomas, 1984), as well as in response to places associated with prior sexual interactions (Bialy, Rydz, & Kaczmarek, 2000). Playback experiments indicate that the production of these vocalizations enhances receptive behaviors of individual estrous females in the presence of devocalized males (McIntosh, Barfield, & Geyer, 1978; White, Cagiano, & Barfield, 1990). Few 22-kHz USVs occur during the appetitive phase of mating, but when they do occur, they have been associated with either female aggression or avoidance of the pursuing male (Anisko, Suer, McClintock, & Adler, 1978; R. Brown, 1979). On the other hand, during the absolute refractory period immediately following ejaculation, when males sit still and do not orient toward the female (van der Poel & Miczek, 1991) and females do not show proceptive behaviors (Sales & Pye, 1974), individual males exclusively emit 22-kHz USVs (Barfield & Geyer, 1975b); they do not emit 50-kHz USVs (McIntosh & Barfield, 1980).

Previously isolated male rats prefer any type of social interaction to none at all but also prefer interaction with a nonaggressive partner to interaction with an aggressive partner (Taylor, 1976). Intermale aggressive episodes involve a mix of aggressive behaviors during attack and either passive or active avoidance during defense. Experimenters commonly elicit intermale aggression in rats within the context of the "intruder paradigm," in which an unfamiliar male intruder is placed into the home cage of a conspecific, which typically attacks the intruder after a brief period of social investigation by both rats. Both 50-kHz USVs and 22-kHz USVs have been observed during this type of encounter (Haney & Miczek, 1993; Sales, 1972), although 22-kHz USVs predominate (van der Poel & Miczek, 1991). Devocalizing manipulations suggest that the intruder emits most of both types of USV (Takahashi, Thomas, & Barfield, 1983). The 50-kHz USVs occur primarily at the outset of an aggressive episode and are associated with aggressive acts such as attacking, boxing, and wrestling (Sales & Pye, 1974). Of interest, 50-kHz USVs also occur when a previously defeated intruder is separated from the resident by a screen (Haney & Miczek, 1993; Tornatzky & Miczek, 1994). However, 50-kHz USVs almost never occur during sustained defeat in aggressive encounters (Thomas et al., 1983). Instead, 22-kHz USVs prevail during submission (Sales & Pye, 1974), particularly when intruder rats adopt sustained crouching or pinned postures (Portavella, Depaulis, & Vergnes, 1993).

In the majority of the complex social interactions described above, 50-kHz USVs coincide with or predict the onset of approach behaviors, whereas 22-kHz USVs coincide with or predict the onset of avoidance behaviors. However, there are two possible exceptions to this pattern. First, in addition to copious 22-kHz USVs, previously defeated male intruders emit some 50-kHz USVs when confronting a resident from across a screen. The appetitiveness or aversiveness of this manipulation has not been demonstrated, but it has traditionally been conceptualized as an aversive, stress-inducing interaction for the defeated male (Tornatzky & Miczek, 1994). However, it is also possible that 50-kHz USVs emitted in this context may reflect a positive affective response to the "safe haven" provided by the experimenter. If so, we would predict that emission of these USVs may predict the degree to which the defeated intruders would exhibit either approach to safety or willingness to affiliate with the resident rat following defeat.

Second, male rats emit 22-kHz USVs during the refractory period following copulation. Again, the appetitiveness or aversiveness of the refractory period has not been examined, but it seems possible that during this period, males no longer find social interaction to be a positive incentive, and indeed may even regard it as aversive. If so, we would predict that during emission of 22-kHz USVs, the male rats would not approach females and vice versa, which appears to be the case (McIntosh & Barfield, 1980). It would be interesting to examine whether the duration of 22-kHz USV production also predicts subsequent latency to approach the female. In addition, because refractory 22-kHz USVs may show more frequency variation than the more monotonic 22-kHz USVs emitted in response to threat stimuli (e.g., defeat, predators), investigators have speculated that they may subserve a somewhat different function (van der Poel & Miczek, 1991). This raises the possibility that these 22-kHz USV subtypes could be fruitfully analyzed as indices of distinct types of negative affect in future research.

Nonsocial Incentive Stimuli

Rats also emit USVs when presented with nonsocial incentive stimuli. For instance, rats approach food sources, and they do so with increasing vigor as a function of the length of food deprivation. Rats that have learned to associate a light cue with a daily feeding opportunity emit 50-kHz but not 22-kHz USVs when the cue is presented prior to feeding, and this effect increases over consecutive days of cue presentation. On the other hand, when trained rats unexpectedly fail to receive food at the end of a cued delay interval, their 50-kHz USVs diminish and they emit 22-kHz USVs instead (Burgdorf, Knutson, & Panksepp, 2000).

Conversely, rats that receive unpredictable footshocks typically show passive avoidance behaviors (e.g., freezing, crouching), as well as active avoidance if an escape route is available (R. J. Blanchard, Dielman, & Blanchard, 1968). Cues that signal impending footshock also elicit 22-kHz USVs (Antoniadis & McDonald, 1999) and decrease emission of 50-kHz USVs in adult rats (Burgdorf et al., 2000). Other nonsocial stimuli that can induce approach or avoidance behavior include administration of electrical brain stimulation and pharmacological compounds as detailed below.

Electrical stimulation of the brain (ESB). Electrical stimulation of specific brain regions can elicit either approach or avoidance behavior. Approach behavior can take an unconditioned form, as in elicitation of approach toward natural rewards in the environment. However, in the absence of appropriate goal objects, unconditioned approach behavior is often difficult to distinguish from general increases in locomotor activity. Thus, investigators often rely on assessment of conditioned approach behaviors such as approach toward stimuli associated with reinforcing ESB. Avoidance behavior can also take both unconditioned forms, as in elicitation of freezing or flight, or conditioned forms, as in avoid-

ance of stimuli associated with electrical shock or aversive ESB (Panksepp, Sacks, Crepeau, & Abbott, 1991).

Investigators have extensively characterized the neural circuitry that evokes unconditioned and conditioned approach behavior in rats with ESB mapping techniques (Panksepp, 1982, 1998). These brain pathways project from dopaminergic nuclei of the midbrain such as the ventral tegmental area (VTA) and substantia nigra and extend upward and medially through the lateral hypothalamus (LH) to the nucleus accumbens (NAcc) and even further into the mesial prefrontal cortex (Stellar & Stellar, 1985). Recent findings indicate that rats make 50-kHz USVs prior to predictable ESB of some of these areas. For instance, rats make 50-kHz USVs but not 22-kHz USVs in an increasing scalloped pattern prior to fixed-interval delivery of ESB to either the VTA or LH (see Figure 3; Burgdorf et al., 2000). Furthermore, cues that predict an impending opportunity to self-administer VTA or LH ESB also evoke 50-kHz USVs but not 22-kHz USVs. Of interest, when ESB is unexpectedly omitted at the end of a cued delay interval as in the case of "frustrative nonreward" (Hug & Amsel, 1969), rats emit fewer 50-kHz USVs but increasing rates of 22-kHz USVs (Burgdorf et al., 2000).

Investigators have also used electrical stimulation to map brain circuits that evoke unconditioned avoidance behaviors, which include freezing at low intensities of stimulation and escape at higher intensities. This circuitry begins in the basolateral amygdala and extends down through the central nucleus of the amygdala, ventral amygdalofugal pathway, and medial hypothalamus to lateral columns of the periaqueductal gray (Panksepp, 1990). Electrical stimulation of some of these areas, such as the posterior hypothalamus and lateral columns of the periaqueductal gray, unconditionally elicits 22-kHz USVs, as does stimulation of areas involved in the modulation of perceived pain intensity such as the medial thalamus (Yajima, Hada, & Yoshii, 1976; Yajima, Hayashi, & Yoshii, 1980; Yajima, Hayashi, & Yoshii, 1981). Although this work on 22-kHz USVs is suggestive, it is unclear to what extent one can generalize from these findings to normal affective states, because subjects were anesthetized and the investigators have not reported on the presence or absence of 50-kHz USVs.

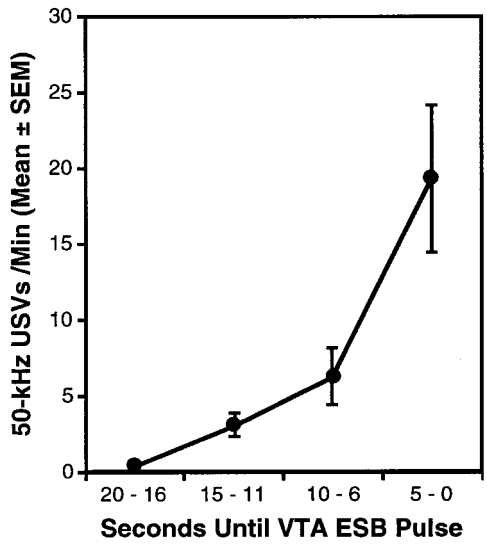

Figure 3. Escalation of 50-kHz ultrasonic vocalizations (USVs) prior to electrical stimulation of the ventral tegmental area (VTA; $n = 9$); taken from Experiment 1 in Burgdorf, Knutson, and Panksepp (2000). ESB = electrical stimulation of the brain.

In sum, although much is known about brain circuitry in which ESB can elicit approach and avoidance behavior in rats, only a few of these regions have been examined in terms of their ability to selectively evoke either 50-kHz USVs or 22-kHz USVs. Presently, electrical stimulation of sites that elicit conditioned approach behaviors (e.g., VTA, LH) can also evoke conditioned 50-kHz USVs, and electrical stimulation of sites that support unconditioned escape behaviors (i.e., the periaqueductal gray) unconditionally elicits 22-kHz USVs. However, many sites remain to be tested or retested, because investigators have not measured both types of USVs in the few relevant experiments that exist. To test affective hypotheses adequately, it will eventually be critical to demonstrate in the same animal that stimulation of sites that unconditionally evoke approach behavior also unconditionally increases 50-kHz USVs, whereas stimulation of sites that unconditionally evoke escape behavior also unconditionally increases 22-kHz USVs.

Pharmacological administration. Peripheral or central administration of many pharmacological compounds can promote approach or avoidance behavior. As with ESB, unconditioned exploratory behaviors commonly include facilitation of approach toward natural rewards. Similarly, conditioned approach behavior can take the form of approach toward stimuli associated with drug administration, including self-administration behavior. Conditioned avoidance can take the form of avoidance of stimuli associated with drug administration. Comparison of the effects of peripheral versus central administration allows investigators to infer whether drugs primarily act through receptors inside or outside of the brain.

Peripheral administration of some pharmacological compounds can unconditionally elicit exploratory behaviors. For instance, peripheral injections of the mixed dopamine agonist amphetamine (AMPH) elicits forward locomotion and sniffing (Robbins & Everitt, 1996), as well as conditioned drug self-administration (Pickens & Harris, 1968). Peripheral injections of AMPH also unconditionally increase emission of 50-kHz USVs in a dose-dependent fashion but generally decrease emission of 22-kHz USVs (Knutson, Burgdorf, & Panksepp, 1997; Wintink & Brudzynski, 2001). This effect can be reversed by peripheral injection of the dopamine antagonist haloperidol (HAL), which blocks AMPH-elicited increases in 50-kHz USVs (Wintink & Brudzynski, 2001). Finally, rats also conditionally emit more 50-kHz USVs but not 22-kHz USVs in places associated with prior AMPH administration (Knutson et al., 1999).

However, peripheral administration of other pharmacological compounds (e.g., nicotine, PCP, opiates, alcohol, cannabis) may not elicit unconditioned exploratory behavior, even though these compounds have the capacity to evoke conditioned self-administration (Wise, 1996). An affective account would predict that administration of these compounds should also conditionally (but not unconditionally) increase 50-kHz USVs. In line with this hypothesis, peripheral morphine (MORPH) injections at high doses, which do not unconditionally facilitate approach behavior, do not unconditionally increase 50-kHz USVs, but cues associated with MORPH administration do (Burgdorf, Knutson, Panksepp, &

Shippenberg, 2001; Knutson et al., 1999; Panksepp & Burgdorf, 2000).

Centrally, most of the pharmacological compounds that reliably promote either unconditioned or conditioned approach behaviors ultimately stimulate dopamine receptors in the NAcc (Schildein, Agmo, Huston, & Schwarting, 1998; Wise & Bozarth, 1987). For instance, central microinjection of AMPH into the NAcc can unconditionally elicit feeding (Swanson, Heath, Stratford, & Kelley, 1997) and can conditionally increase appetitive behaviors as well (Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999). Accordingly, microinjection of AMPH into the NAcc also unconditionally and dose dependently increases 50-kHz USVs but not 22-kHz USVs (Burgdorf, Knutson, Panksepp, & Ikemoto, 2001). Central microinjection of AMPH or glutamate into the medial preoptic area of the hypothalamus (MPOA) also unconditionally increases appetitive sexual behaviors, whereas microinjection of dopamine antagonists into this region halts these appetitive behaviors and evokes freezing instead (Moses, Loucks, Watson, Matuszewich, & Hull, 1995; Pehek et al., 1988). Microinjection of both AMPH and glutamate into the MPOA also unconditionally evokes 50-kHz USVs, and peripheral administration of the dopamine antagonist HAL blocks these effects (Wintink & Brudzynski, 1999).

Peripheral administration of other pharmacological compounds can evoke unconditioned and conditioned avoidance behaviors. For instance, both the opiate antagonist naloxone (NAL) as well as lithium chloride (LiCl) reliably elicit both unconditioned and conditioned avoidance behaviors in rats (Tzschentke, 1998). Peripheral injection of NAL unconditionally increases 22-kHz USVs and decreases 50-kHz USVs, and cues associated with NAL administration also conditionally increase 22-kHz and decrease 50-kHz USVs (Burgdorf, Knutson, Panksepp, & Shippenberg, 2001). Although the unconditional effects of peripheral injections of LiCl have yet to be examined, cues associated with LiCl administration conditionally increase 22-kHz USVs and decrease 50-kHz USVs (Burgdorf, Knutson, Panksepp, & Shippenberg, 2001). Researchers have also recorded unconditioned USVs during withdrawal from approach-eliciting substances. Consistent with an opponent-process model of the affective processes underlying drug addiction (Solomon & Corbit, 1974), rats emit more 22-kHz USVs in response to startling stimuli presented during withdrawal from self-administered compounds (including cocaine, Barros & Miczek, 1996; Mutschler & Miczek, 1998, morphine, Vivian & Miczek, 1991, and diazepam, Miczek & Vivian, 1993; Vivian, Farrell, Sapperstein, & Miczek, 1994) than when not undergoing withdrawal.

Central administration of pharmacological compounds can also evoke unconditioned aversive behaviors. For instance, microinjection of compounds that stimulate acetylcholine receptors (e.g., the muscarinic agonist carbachol) along the ascending trajectory of the cholinergic neurons of the lateral dorsal tegmental (LDT) nucleus of the midbrain unconditionally elicits freezing and crouching in adult rats (Brudzynski, McLachlan, & Girvin, 1989; Brudzynski & Mogenson, 1986). Either glutamatergic activation of these LDT cholinergic neurons or cholinergic microinjections along their ascending trajectory (including medial hypothalamic nuclei such as the MPOA and the medial septum) also unconditionally evoke 22-kHz USVs, an effect that can be reversed by peripheral injection of cholinergic antagonists (Brudzynski, 2001). Twenty-two kilohertz USVs elicited by pharmacological injections are sonographically indistinguishable from those elicited by nonpharmacological stimuli (e.g., touch by an unfamiliar experimenter or foot shock; Brudzynski, Ociepa, & Bihari, 1991). In addition, microinjection of excitatory amino acids into caudal regions of the lateral column of the periaqueductal gray of the midbrain powerfully elicits a combination of active escape behavior followed by freezing, accompanied by autonomic and cardiac arousal. Consistent with ESB experiments, this manipulation also elicits 22–28 kHz USVs in awake rats in both social and nonsocial contexts (Carrive, 1993; Depaulis, Keay, & Bandler, 1992).

Investigators have also examined unconditioned and conditioned effects of many other pharmacological compounds on USVs. However, we do not include those findings here because the capacity of some of these compounds to elicit approach or avoidance behavior has not been adequately evaluated in rats. In addition, many of these pharmacological manipulations occurred in the context of social encounters that may elicit combinations of approach and avoidance behaviors, thus creating scenarios with relatively ambiguous blends of incentive demands (e.g., the effects of gepirone on USVs during aggression; Vivian & Miczek, 1993).

In summary, administration of pharmacological compounds that evoke unconditioned exploration (e.g., peripheral or central AMPH) also unconditionally elicits 50-kHz USVs, whereas cues associated with compounds that evoke conditioned approach (e.g., peripheral MORPH) conditionally elicit 50-kHz USVs. On the other hand, acute administration of compounds that elicit unconditioned freezing or escape (e.g., peripheral NAL, central carbachol) also unconditionally elicits 22-kHz USVs, whereas cues associated with compounds that promote conditioned avoidance behaviors (e.g., peripheral NAL and LiCl) conditionally increase 22-kHz USVs and decrease 50-kHz USVs. Finally, physiological withdrawal from pharmacological compounds that acutely elicit conditioned approach (e.g., peripheral MORPH) increases startle-elicited 22-kHz USVs in adult rats.

Affective Overview

Consistent with the affective account, in both social and nonsocial circumstances, stimuli that evoke approach also elicit 50-kHz USVs, whereas stimuli that evoke avoidance elicit 22-kHz USVs (see Table 1). In no cases have researchers reported that 50-kHz USVs occur during freezing behaviors or 22-kHz USVs occur during approach behaviors. Of interest, conditioning experiments indicate that rats reliably produce USVs in response to cues that predict rewards or punishments, implying that primary incentives are not required for their production. Thus, USVs may occur as a function of rats' anticipation of rewards or punishments (Burgdorf et al., 2000; Knutson, Burgdorf, & Panksepp, 1998). Indeed, in some cases, prior emission of USVs in response to presentation of a stimulus predicts approach behavior directed toward that stimulus (Burgdorf & Panksepp, 2001; Knutson, Burgdorf, & Panksepp, 1998). We now turn to an assessment of the relative explanatory power of the mechanical by-product and social signal accounts of USV production.

Table 1
Effects of Appetitive and Aversive Incentive Stimuli on Rat Ultrasonic Vocalizations

Stimulus	50 kHz (short)	22 kHz (long)	Source
		Social	
Appetitive			
Presence of estrous female	+	o	Geyer et al. (1978)
Estrous female odor	+	o	Geyer et al. (1978)
Conspecific play	+	o	Knutson, Burgdorf, & Panksepp (1998)
Presence of play partner	+	o	Knutson, Burgdorf, & Panksepp (1998)
Play place cue	+	o	Knutson, Burgdorf, & Panksepp (1998)
After play deprivation	+	o	Knutson, Burgdorf, & Panksepp (1998)
Tickling by familiar human	+	o	Panksepp & Burgdorf (1999)
Tickling cue	+	o	Panksepp & Burgdorf (1999)
Aversive			
Presence of predator	?	+	R. J. Blanchard et al. (1991)
Touch by unfamiliar human	o	+	Brudzynski & Ociepa (1992)
Predator odor	−	?	Panksepp & Burgdorf (1999)
		Nonsocial	
Appetitive			
Food cue	+	o	Burgdorf et al. (2000)
Aversive			
Bright light in play chamber	−	o	Knutson et al. (1998)
Footshock cue	?	+	Antoniadis & McDonald (1999)
Footshock cue	−	+	Burgdorf et al. (2000)
Food cue extinction	−	+	Burgdorf et al. (2000)
		ESB	
Appetitive			
Prior to fixed VTA ESB	+	o	Burgdorf et al. (2000)
Prior to fixed LH ESB	+	o	Burgdorf et al. (2000)
VTA ESB self-administration cue	+	o	Burgdorf et al. (2000)
LH ESB self-administration cue	+	o	Burgdorf et al. (2000)
Aversive			
Medial thalamic ESB	o	+	Yajima et al. (1976)
Posterior hypothalamic ESB	?	+	Yajima et al. (1980)
PAG ESB	?	+	Yajima et al. (1980)
VTA ESB self-administration extinction	−	+	Burgdorf et al. (2000)
LH ESB self-administration extinction	−	+	Burgdorf et al. (2000)
		Pharmacological	
Appetitive			
AMPH place cue	+	o	Knutson et al. (1999)
MORPH place cue	+	o	Knutson et al. (1999)
MORPH place cue	+	o	Burgdorf, Knutson, Panksepp, & Shippenberg (2001)
Peripheral AMPH	+	o	Knutson et al. (1997)
Peripheral AMPH	+	?	Panksepp & Burgdorf (2000)
Peripheral AMPH	+	o	Wintink & Brudzynski (2001)
Central AMPH (NAcc)	+	o	Burgdorf et al. (2001)
Central AMPH (MPOA)	+	o	Wintink & Brudzynski (1999)
Central glutamate (MPOA)	+	o	Fu & Brudzynski (1994)
Aversive			
NAL place cue	−	+	Burgdorf et al. (2000)
LiCl place cue	−	+	Burgdorf et al. (2000)
Peripheral NAL	−	+	Burgdorf, Knutson, Panksepp, & Ikemoto (2001)
Central carbachol (MPOA)	o	+	Fu & Brudzynski (1994)
Central carbachol (LS)	o	+	Brudzynski (2001)
Central carbachol (AH)	o	+	Brudzynski (2001)
Central glutamate (LDT)	o	+	Brudzynski (2001)
Central glutamate (PAG)	?	+	Depaulis et al. (1992)

Note. + = increase; − = decrease; o = no change; ? = not measured; VTA = ventral tegmental area; ESB = electrical stimulation of the brain; LH = lateral hypothalamus; PAG = periaqueductal gray; AMPH = amphetamine; MORPH = morphine; NAcc = nucleus accumbens; MPOA = medial preoptic area of the hypothalamus; NAL = naloxone; LiCl = lithium chloride; LS = lateral septum; AH = anterior hypothalamus; LDT = lateral dorsal tegmentum.

Alternate Hypotheses Regarding USV Function

Mechanical By-Product Hypotheses

Motor artifact. On the basis of ethological studies of gerbils (Thiessen, Kittrell, & Graham, 1980), it has been proposed that high-frequency USVs may be a by-product of locomotor activity. According to an elaboration of this theory, rat 50-kHz USVs result from air expulsion following thoracic compression induced by forepaw impact on hard surfaces (Blumberg, 1992). Although both gerbils and rats belong to the same suborder *Myomorpha* of the order *Rodentia,* generalizing from the USVs of rats (family: *Muridae*) to those of gerbils (family: *Cricetidae*) may be problematic. Generally, rat USVs feature clearer harmonic components of narrower bandwidth than do gerbil USVs (Sales & Pye, 1974). In addition, the ultrasound detectors used in studies of gerbils may not have been set to optimally distinguish USVs from noise transients caused by forepaw contact with the ground (which should lack harmonic structure and a constrained bandwidth). Although adult male gerbils typically vocalize in the 35–65 kHz range (Sales & Pye, 1974), Thiessen et al. used detectors set to 33 ± 5 kHz and so may have detected fewer high-frequency USVs and more noise transients than they had intended. Nonetheless, in these studies, devocalization via laryngeal nerve section did transiently diminish the rates of USVs detected. Further, the apparent coincidence of high levels of locomotor activity and 50-kHz USVs during vigorous approach behavior in adult rats could be taken to support the motor artifact hypothesis.

However, several lines of evidence suggest that although they may often coincide with locomotor activity, 50-kHz USVs are not merely a by-product of locomotor activity. First, in experiments in which both 50-kHz USVs and activity have been concurrently measured, changes in locomotor activity cannot statistically account for changes in 50-kHz USVs. For instance, although delivery of rewarding ESB increases both 50-kHz USVs and locomotor activity (as measured by quadrant entries), covariance analyses indicate that ESB-induced increases in activity cannot statistically account for increases in 50-kHz USVs. However, increases in 50-kHz USVs can statistically account for increased locomotor activity (Burgdorf et al., 2000).

Second, production of 50-kHz USVs and locomotor activity can be experimentally dissociated. For example, play-experienced juvenile rats emit many more 50-kHz USVs while separated from a potential partner prior to a play session than during the play session itself, despite the fact that they show more locomotor activity during play (as measured by a stabilimeter; Knutson, Burgdorf, & Panksepp, 1998). In a second example, rats emitted increasing levels of 50-kHz USVs during daily presentation of a light cue associated with impending rewarding ESB, but activity (as measured by quadrant entries) remained constant (Burgdorf et al., 2000). In a third example, rats emitted more 50-kHz USVs after microinjection of AMPH into the shell of the NAcc versus the core of the NAcc, even though both manipulations evoked indistinguishable levels of locomotor activity (as measured by photocell counts of vertical and horizontal movements; Burgdorf, Knutson, Panksepp, & Ikemoto, 2001).

Third, 50-kHz USVs can predict specific types of locomotor activity that take the form of approach behavior. For instance, in juvenile rats at play, 50-kHz USVs on a given day predicted play solicitations on following days (as measured by dorsal contacts), but not the reverse (Knutson, Burgdorf, & Panksepp, 1998). Moreover, 50-kHz USVs predict how rapidly rats approach a cue associated with prior play with a familiar human (Burgdorf & Panksepp, 2001). On the other hand, cases in which locomotor activity predicts subsequent 50-kHz USVs have not been documented.

Together, this evidence suggests that 50-kHz USVs are not merely a by-product of locomotor activity. Instead, the affective account would predict that 50-kHz USVs are more tightly correlated with appetitive behaviors invoked by reward anticipation than with other types of activity. Future studies will have to address this prediction using fine-grained analyses of specific behaviors. Still, stimuli that predict reward can elicit 50-kHz USVs, even when approach is not possible (Burgdorf, Knutson, Panksepp, & Ikemoto, 2001). Unlike the motor artifact hypothesis, the affective account implies that presentation of different types of incentive stimuli will elicit different types of USVs, even when motor activity is held constant by either statistical or physical means.

Temperature regulation. Changes in USV emission might result not only from externally visible changes in locomotor activity but also from internal homeostatic changes, such as those due to thermoregulation (Zippelius & Schleidt, 1956). This hypothesis accords with research demonstrating that infant rats develop their limited abilities to regulate their own body temperature from 4–16 days of age. During this stage of development, they also show increased "distress calls" upon lowering of the ambient temperature (< 25°C; Allin & Banks, 1971; Blumberg & Sokoloff, 2001; Okon, 1971). However, aside from the fact that adult rats do regulate their own body temperature and external temperature was kept constant across all conditions in all of the studies cited, experimental manipulations that induce similar changes in body temperature elicit divergent types of USVs in adult rats. For instance, cues associated with food delivery (an appetitive incentive) and electric shock (an aversive incentive) both elicit increases in body temperature (Cunningham & Schwarz, 1989). However, whereas food cues increase 50-kHz USVs (Burgdorf et al., 2000), shock cues instead increase 22-kHz USVs and decrease 50-kHz USVs (Antoniadis & McDonald, 1999; Burgdorf et al., 2000). This dissociation suggests that although body temperature may provide a useful index of general arousal in adult rats, it is unlikely to distinguish between appetitive and aversive forms of arousal as do different types of USVs.

Social Signal Hypotheses

Other investigators have proposed that both 50- and 22-kHz USVs serve as social signals (Barfield & Thomas, 1986; R. J. Blanchard et al., 1991), and ample evidence exists to support these claims. For instance, playback of 50-kHz USVs enhances receptive behaviors in female rats during mating (Thomas, Howard, & Barfield, 1982), whereas playback of 22-kHz USVs induces freezing and passive avoidance behaviors in adult rats (Brudzynski & Chiu, 1995; Sales, 1991).

Although USVs do appear to serve social functions in different types of interactions, they may communicate information pertaining more to the affective state of the sender than to the propositional content of the situation per se. In other words, when 22-kHz

USVs occur in different situations, they tend to occur in the context of impending punishment and so may signal anticipation of punishment on the part of the vocalizer. Conversely, when 50-kHz USVs occur in different situations, they tend to occur in the context of impending reward and so may signal anticipation of reward on the part of the vocalizer. In ethological parlance, an affective account posits that different types of rat USVs carry motivational but not necessarily referential information (Marler, Evans, & Hauser, 1992). An affective account thus parsimoniously predicts that similar types of USVs (e.g., 22-kHz USVs) may occur in distinct, yet thematically related, situations (e.g., defeat, anticipation of shock; van der Poel & Miczek, 1991).

An alternative ethological account posits that vocalizations can directly induce affect in conspecifics rather than representing information about a sender's affective state (Owren & Rendall, 1997). According to this account, affect induction in conspecifics may occur either unconditionally, on the basis of a vocalization's pleasant or aversive qualities, or conditionally, by preceding pleasant or aversive outcomes. However, this account's proposed mapping of specific vocalization characteristics to unconditional affective inductions may need some revision in the case of adult rats. Specifically, the affect-induction model predicts that subordinate mammals should emit vocalizations of high frequency, loud amplitude, and noisy spectral qualities to unconditionally induce an NA-like state in dominant conspecifics. However, the ethological evidence suggests the opposite in the case of adult rats. Playback of relatively low (22-kHz) vocalizations are more likely to evoke behaviors that are associated with NA such as freezing in conspecifics, whereas playback of relatively high (50-kHz) vocalizations are instead likely to induce behavior associated with PA such as approach (Brudzynski & Chiu, 1995; Sales, 1991; Thomas et al., 1982). Nonetheless, the affective and affect-induction accounts of vocalization function need not be mutually exclusive—both could be true. However, here we argue that affective experience (as indexed by arousal and subsequent approach or avoidance behavior) is a necessary feature for emission of USVs in adult rats. If affect does not occur in the sender, then we predict that USVs will not occur.

Even if USVs do serve social functions such as communicating affective experience or inducing affect in conspecifics, the existing evidence suggests that social stimuli are not required for their elicitation. As detailed earlier, nonsocial rewards and punishments can elicit USVs (e.g., food, shock, administration of brain stimulation, and injection of pharmacological compounds). Thus, social interaction is not a necessary condition for eliciting USVs in adult rats, although it can powerfully evoke and modulate USV production. For instance, the presence of conspecifics robustly enhances production of 22-kHz USVs in response the presence of a predator (R. J. Blanchard et al., 1991). Future research will have to more systematically determine under what conditions and to what extent social stimuli modulate USV production.

Methodological and Conceptual Considerations

Measurement

Even if USVs index affective states in rats, work on the functional characterization of different types of USVs has just begun, and several methodological issues threaten to limit the practical utility of these measures. First, as noted earlier, rats tend to emit different types of USVs at different developmental stages. Although no formal developmental study of 50-kHz USVs or 22-kHz USVs exists, emission of 50-kHz USVs appears to peak during juvenile development, whereas emission of 22-kHz USVs follows a more constant time course over rats' life span (Panksepp et al., 1998).

Second, adult rats show marked individual differences in their tendency to produce USVs. These individual differences show some degree of stability over time. For instance, in a study of playing juvenile rats, investigators have reported an average correlation of .67 for the expression of 50-kHz USVs within the same dyad across 5 days of play with a conspecific (Knutson, Burgdorf, & Panksepp, 1998) and an average correlation of .65 for the same individual across 4 days of play with an experimenter (Burgdorf & Panksepp, 2001). However, the extent to which the same individual expresses reliable levels of either 50- or 22-kHz USVs in other situations has yet to be determined. Extensive work has also shown reliable individual differences in infant rats' production of 40-kHz USVs. These USVs show stable individual differences across assessments at 10 and 15 days of age on the order of $r = .51$ (Brunelli, Keating, Hamilton, & Hofer, 1996). Individual differences in the production of 40-kHz USVs during infancy also predict rats' proclivity to show fear-related behaviors in later adulthood (Dichter, Brunelli, & Hofer, 1996). However, researchers have not yet examined the cross-temporal stability of 22-kHz USV production. In addition, research has not elucidated whether the tendency to make 50-kHz USVs correlates with the tendency to make 22-kHz USVs or not in adults, nor the extent to which social rearing conditions influence individual differences in adult USV production.

As a third measurement issue, USV production often changes as a function of repeated exposure to the same stimulus or situation. For instance, when first introduced to a novel environment, rats tend to make either no USVs or a few 22-kHz USVs, which correlate with freezing behavior. Upon subsequent reintroductions to the same environment, rats emit occasional 50-kHz USVs, and the expression of these vocalizations appears to correlate with exploratory behaviors. After many reintroductions, rats again make fewer USVs (Knutson, Burgdorf, & Panksepp, 1998). Future research will doubtless determine the distinct roles of habituation, sensitization, and learning in modulating the emission of USVs during repeated exposures to the same stimulus. None of the above issues (i.e., developmental trajectory, individual differences, modulation by learning) argue against an affective interpretation of USVs, but they do illustrate some factors that may complicate measurement attempts.

Finally, measures of some USVs (i.e., particularly 50-kHz USVs) have proven difficult to automate, because ultrasonic detectors sometimes register false positives from unrelated transient noises in the environment that may carry partials in the ultrasonic frequency range. Nonetheless, trained human coders can detect and distinguish different types of USVs online with ultrasonic detectors or on downsampled audio recordings. By using specific frequency range and sound length criteria, these coders have been able to attain high interrater agreement (Brunelli et al., 1996; Burgdorf, Knutson, Panksepp, & Shippenberg, 2001; Knutson, Burgdorf, & Panksepp, 1998). Ultimately, however, such detection work must be validated with more rigorous sonographic recording

techniques (Brudzynski, 2001; Wintink & Brudzynski, 2001). In addition to becoming the "industry standard" in future studies of rat USVs, sonographic analysis may well reveal additional functional USV subtypes that are difficult to distinguish with human auditory coding techniques.

Mechanism

A well-established literature suggests that rats and other rodents are specifically prepared to both produce and perceive USVs (A. M. Brown & Pye, 1975; Roberts, 1975). Transection studies indicate that the integrity of the laryngeal nerve is essential for the production of both USVs and audible vocalizations (Roberts, 1975). Electrophysiological studies indicate that neurons at the root of the laryngeal nerve in the nucleus ambiguous of the medulla show selective responses as much as 80 ms prior to and during the production of 22-kHz USVs. These neurons do not necessarily respond during production of audible vocalizations, inspiration, or exhalation alone (Yajima & Hayashi, 1983). However, even though rodents use the larynx to produce both audible vocalizations and USVs, different laryngeal mechanisms may underlie the production of each type of vocalization. Studies of rat sound production in light gases (e.g., helium) indicate that whereas rats use vibrating structures of the larynx (i.e., the vocal folds) to produce audible vocalizations (as is true of most other mammals), they instead produce USVs with a distinct whistlelike laryngeal mechanism (Roberts, 1975). The use of such laryngeal mechanisms helps to explain why USVs usually involve primarily pure tones, whereas audible rat vocalizations carry a more complex harmonic structure.

In addition to specialized physiological methods of producing USVs, rats also have specialized physiological proclivities for perceiving USVs. Most rodents show peaks of sensitivity at the level of both the cochlear membrane and the inferior colliculus to sounds in the ultrasonic range, and these peaks vary somewhat between but not within species (A. M. Brown, 1976). In laboratory rats, cochlear membrane recording studies have indicated dual ultrasonic sensitivity peaks near 20 kHz and 40 kHz (Crowley, Hepp-Reymond, Tabowitz, & Palin, 1965), and inferior colliculus electrophysiological studies show similar bimodal sensitivity peaks (A. M. Brown, 1973). The observation of distinct sensitivity peaks at these early levels of sound processing suggests that rats are prepared to detect certain sounds at specific ultrasonic frequencies. The correspondence of these sensitivity peaks with the natural tendency of rats to vocalize in the 18–70 kHz range is probably not coincidental (A. M. Brown, 1976). Of interest, rodents' auditory sensitivity to different ultrasonic frequencies may vary according to age and incentive demands. For example, in mice, Markl and Ehret (1973) demonstrated a sensitivity peak at 50 kHz, in addition to a peak at 20 kHz, only when subjects were rewarded for a correct discrimination response, not when they were punished for incorrect responses (Markl & Ehret, 1973). Studies of this type remain to be conducted on the rat.

In addition to enhanced sensitivity for ultrasonic frequencies at more basic levels of auditory processing, rodents also have specialized cortical regions dedicated to higher order processing of ultrasonic sounds. These specialized cortical fields have been extensively mapped in the house mouse, *Mus musculus* (Stiebler, Neulist, Fichtel, & Ehret, 1997). Relative to primary auditory fields, ultrasonic fields are located more rostrally and dorsally, with certain rostral regions responding optimally to frequency-modulated sounds in the 40–80 kHz range (Hofstetter & Ehret, 1992). Unlike primary auditory fields, ultrasonic fields lack tonotopic organization, suggesting that they subserve a categorical perceptual function, which has been borne out by behavioral choice studies in mice (Ehret & Haack, 1981). Cortical ultrasonic fields have also been identified, but not characterized as extensively, in laboratory rats (Horikawa, Ito, Hosokawa, Homma, & Murata, 1988). Together with the evidence for specialized ultrasound production mechanisms, the existence of specialized ultrasound perceptual mechanisms implies that rats, like other mammals, come evolutionarily prepared to communicate their affective experience to conspecifics.

Generalizability

We have argued that USVs may index affective states in rats. Although such a hypothesis runs the risk of unwarranted anthropomorphism (Blumberg & Sokoloff, 2001; Panksepp, in press), we believe that this risk is justified by the potential benefits of understanding the brain substrates of affective experience in mammals (Panksepp, 1998). Nonetheless, care must be taken in specifying exactly how the present hypothesis might or might not generalize to other species, including humans.

Of all rodents (order: *Rodentia*), USVs have not been observed in the suborders *Hystricomorpha* (porcupines and guinea pigs) or *Scuriomorpha* (squirrels and beavers) but have been documented in the suborder *Myomorpha*, including both the subfamilies *Muridae* (rats and mice) and *Cricetidae* (gerbils, hamsters, voles, and lemmings; Sales & Pye, 1974). Although smaller rodent species are more likely to emit USVs than larger species (Blumberg & Sokoloff, 2001), the fact that adult rats can produce higher frequency USVs than those of infant rats argues against the simple hypothesis that vocalization frequency is merely a linear function of larynx size. Because they belong to the same subfamily, and abundant research has been done on the functional properties of their USVs, mice provide an appropriate test case of the generalizability of the affective expression hypothesis in a rodent other than rats.

Typically, mice exhibit high short USVs ranging from 40–110 kHz with a mean frequency of 70 kHz (Sales & Pye, 1974), which may correspond to rats' 50-kHz USVs because of the smaller size of the murine larynx. Generally, these vocalizations have been studied in the context of sexual activity, and presentation of female pheromones can readily evoke these vocalizations in males (Nyby & Whitney, 1978). More recent work also suggests that visual cues and social history can also modulate the expression of these vocalizations (Nunez & Tan, 1984; Sipos, Wysocki, Nyby, Wysocki, & Nemura, 1995). Although 70-kHz USVs have typically been deemed an index of courtship (D'Amato, 1991) or male sexual arousal (Dizinno, Whitney, & Nyby, 1978), some evidence suggests that they reflect more general appetitive processes, because they occur in anticipation of sexual activity (White, Prasad, Barfield, & Nyby, 1998), as well as in nonsexual contexts. For instance, 70-kHz USVs occur in the context of female–female social encounters, particularly when one animal is carrying food cues (Moles & D'Amato, 2000). As Bean and colleagues suggested, 70-kHz USVs may serve the social function of "attract(ing)

other mice" (Bean, Nunez, & Wysocki, 1986, p. 51). Thus, in mice, the 70-kHz USV appears to reflect a desire for positive social interactions, and perhaps other rewards, but the manner in which this USV is modulated by nonsocial rewards has not yet received extensive characterization. Nonetheless, existing work does suggest some functional generalizability between murine 70-kHz USVs and rat 50-kHz USVs.

On the other hand, there is currently little empirical evidence for the occurrence of a murine 40-kHz USV that subserves a function analogous to rat 22-kHz USVs. For instance, whereas rats emit 22-kHz USVs in response to the presence of a predator such as a cat, mice do not (D. C. Blanchard, Griebel, & Blanchard, 2001), even though both show avoidance behavior. Theorists have postulated that mice do not show threat-related USVs because they do not live in multimale groups that necessitate negotiation of dominance hierarchies (D. C. Blanchard et al., 2001). However, future work must examine murine responses to other nonsocial threat stimuli (e.g., aversive ESB) before the existence of such a vocalization can be ruled out.

In rats, we have argued that 50-kHz USVs indicate but do not constitute PA—a dynamic neural state that is not yet directly observable (Bollen, 1989). Others have persuasively used freezing, defecation, and even USVs to index NA, or fear-related processes in rats (Antoniadis & McDonald, 1999; Fendt & Fanselow, 1999; LeDoux, 1993). Although more distantly related humans do not emit USVs, they do show other unconditioned nonverbal behaviors that putatively index affective states. These behaviors include (but are not limited to) facial expressions and changes in vocal prosody. Specifically, experiments on facial muscular movement indicate that unconditioned activity of the zygomatic major (upper cheek) muscle correlates with self-reported PA while viewing slides with emotional content, whereas activity of the corrugator supercilii (brow) correlates with self-reported NA (Lang et al., 1990).

Surprisingly, experimental studies of human vocal expression have identified clear prosodic markers of affective arousal, but not yet of valence (i.e., PA vs. NA; Scherer, 1995). Although it seems possible that certain vocalizations indicate PA (e.g., laughter) whereas others indicate NA (e.g., crying), the physical acoustic features that would enable humans to identify and discriminate such highly variable vocalizations have not yet been characterized (Bachorowski, Smoski, & Owren, 2001). Given the punctate timing and high frequency of rats' 50-kHz USVs, it is tempting to hypothesize not only that they index a state similar to human PA but also that they spring from the workings of a structurally similar mechanism to that which produces laughter in humans (Wiley, 1981). Along these lines, Panksepp and Burgdorf proposed that the 50-kHz USVs of rats are regulated by a homologous brain mechanism that promotes play-induced laughter in human children (Panksepp & Burgdorf, 1999).

However, the specialized nature of rat mechanisms for rat USV production and perception makes it unlikely that they are structurally homologous to human laughter or any other audible vocalizations. As mentioned earlier, rats produce USVs through a whistle-like laryngeal mechanism, whereas humans vocalize by vibrating the vocal folds (Roberts, 1975). On the other hand, some species of nonhuman primates make acoustically distinct audible vocalizations during anticipation of reward versus punishment (Jurgens, 1998). Extensive brain mapping studies have revealed that stimulation of similar brain regions that elicit "affective" USVs in rats also unconditionally elicit "affective" audible vocalizations in squirrel monkeys. For example, NAcc ESB elicits vocalizations associated with appetitive behavior, whereas medial hypothalamic ESB elicits vocalizations associated with aversive behavior (Jurgens, 1998).

A more conservative interpretation of these collective findings is that in rats, 50-kHz USVs index a state that is functionally analogous to human PA. Whether or not 50-kHz USVs are generated by a mechanism that is structurally homologous to the mechanism that produces certain human vocalizations remains unclear. Perhaps ironically, the research that could best help to resolve this issue must come from the realm of human neuroethological studies, because functional aspects of human laughter have not been well-characterized. For instance, no research has addressed whether human laughter or other vocalizations are likely to occur either during anticipation or consumption of rewards (Panksepp, 2000; Provine, 2001). In addition, no research has addressed whether laughter is more likely to occur during an increase in PA, sudden relief from NA, both, or neither (Panksepp, Knutson, & Burgdorf, 2002). Some empirical research does suggest that both "felt" smiling and laughter correlate with higher levels of self-reported PA evoked by humorous stimuli (Ruch, 1997), but it is unclear whether PA precedes laughter or vice versa. In summary, although rats and humans may have functionally analogous affective experiences, the existing literature does not yet support the notion that they have structurally homologous modes of affective expression.

Implications

If USVs index affective states in rats, then they could serve as a means of modeling affective experience and hedonic preference in other mammals, including humans. Unconditionally elicited USVs may provide a useful tool for localizing the neural substrates of unconditioned affective experience itself. For instance, AMPH injection into the NAcc (a part of the ventral striatum) but not the caudate (a part of the dorsal striatum) unconditionally elicits 50-kHz USVs but not 22-kHz USVs (Burgdorf, Knutson, Panksepp, & Ikemoto, 2001). Investigators could use electrical stimulation or microinjection techniques to explore whether USVs can be unconditionally elicited from other brain sites. We would predict that stimulation of sites that unconditionally evoke 50-kHz USVs should also unconditionally promote approach behavior, whereas stimulation of sites that unconditionally evoke 22-kHz USVs should unconditionally promote avoidance behavior, even in the absence of appropriate external cues (Brudzynski, 2001). Given the relative independence of PA and NA in human self-report indices, we might expect brain pathways associated with PA and NA (and even subsets of these pathways) to be neuroanatomically and neurochemically distinguishable (Knutson, Wolkowitz, et al., 1998; Panksepp, 1998; Panksepp et al., 1998).

On the other hand, conditionally elicited USVs might provide a predictive index of hedonic preference. For instance, rats might prefer both stimuli that increase PA and stimuli that reduce NA. As a case in point, whereas peripheral AMPH both unconditionally and conditionally elicits 50-kHz USVs, peripheral MORPH primarily conditionally elicits 50-kHz USVs (Knutson et al., 1999; Panksepp & Burgdorf, 2000). Thus, whereas AMPH may act more directly on brain substrates that increase PA as defined earlier (i.e.,

anticipation of reward plus arousal), MORPH may have a less direct effect on pathways implicated in PA or may more powerfully initially act on pathways that reduce NA (Panksepp et al., 2002). However, if rats come to prefer both AMPH and MORPH, we would predict that rats should conditionally emit 50-kHz USVs when presented with cues that predict administration of either of these pharmacological agents.

As an index of hedonic preference, conditioned 50-kHz USVs might serve as a rapid screen of potential abuse liability in novel psychoactive compounds, augmenting currently popular paradigms such as conditioned place preference and self-administration (Bardo & Bevins, 2000; Calcagnetti & Schechter, 1992; Knutson et al., 1999; Schuster & Thompson, 1969; Tzschentke, 1998). On the other hand, conditioned 22-kHz USVs might alert investigators to potentially aversive side effects of pharmacological compounds (Burgdorf, Knutson, Panksepp, & Shippenberg, 2001). In addition, as previously suggested by others (Miczek, Weerts, Vivian, & Barros, 1995), unconditional reduction of threat-induced 22-kHz USVs might index a compound's ability to reduce NA or distress. If distinct neural circuits govern the experience of PA versus NA, then a compound's abuse liability should not necessarily predict its ability to relieve distress, and vice versa (Panksepp et al., 2002).

Should they prove reliable, individual differences in USV production may also serve as a phenotypic marker of individual variation in affective experience. Well-controlled studies have already demonstrated the reliability and predictive validity of infant 40-kHz USVs in rats, because they predict fearful behaviors in adulthood (Brunelli & Hofer, 1996; Dichter et al., 1996). We would predict that adult rats that produce high levels of 22-kHz USVs in response to threat cues will have more severe physiological reactions to stressful events, potentially providing a model of humans with high dispositional NA (Watson et al., 1999). Longitudinal studies have demonstrated that these individuals have a higher vulnerability to develop unipolar depression and anxiety disorders (Krueger, Caspi, Moffitt, Silva, & McGee, 1996). On the other hand, rats that produce high levels of 50-kHz USVs in response to reward cues may provide a model of humans with high dispositional PA. Indeed, breeding for 50-kHz tickle-induced USVs has already indicated that rats with the highest vocalization rate play the most (Panksepp, Burgdorf, & Gordon, 2001). These phenotypes might also help investigators to identify, isolate, and manipulate genetic protein expression mechanisms that contribute to heritable differences in affective style (Bond, 2001; Bouchard, 1994; Knutson, Burgdorf, & Panksepp, 1998).

Summary

In summary, we have presented the hypothesis that 50-kHz USVs and 22-kHz USVs differentially index positively and negatively activated affective states in adult rats. We predicted that if these USVs indexed PA and NA, then they would occur prior to or during the initial appearance of approach or avoidance behavior, respectively. Ethological, pharmacological, and brain stimulation findings support this hypothesis. Existing evidence also suggests that peripheral physiological mechanisms such as locomotor activity and temperature regulation can be dissociated from USV emission in adult rats and thus cannot fully account for their incidence. Further, although USVs commonly occur in social contexts, social contact is not an essential requisite for their production, because adult rats emit USVs even in the presence of nonsocial incentive stimuli. The degree to which social and other types of conditioning during early development may influence adult production of USVs remains to be examined.

Much work remains to be done to establish the generalizability of this thesis to complex ethological scenarios, to different rat strains, and to other rodent species. Additional research must also elucidate whether the arousal component of PA and NA must invariably co-occur with USV emission. Nonetheless, overall, the data presented here do suggest that 50- and 22-kHz USVs index positive and negative activated affective states in rats. In addition to providing a novel tool for modeling human affective experience and hedonic preference, this simple hypothesis illustrates the mutual benefit that can be derived from exploring human psychological models of affective function with comparative neuroscience approaches.

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