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Research report

Harmonic and frequency modulated ultrasonic vocalizations reveal differences in conditioned and unconditioned reward processing

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HIGHLIGHTS

- Rat novelty and sensation seeking is complex and requires more than one measure.
- FM and harmonic USVs change differently with repeated tickling.
- The change in harmonic USVs was positively related with novelty seeking.
- FM and harmonic USVs can be used to understand attribution of incentive value.

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ABSTRACT

Novelty and sensation seeking (NSS) and ultrasonic vocalizations (USVs) are both used as measures of individual differences in reward sensitivity in rodent models. High responders in the inescapable novelty screen have a greater response to low doses of amphetamine and acquire self-administration more rapidly, while the novelty place preference screen is positively correlated with compulsive drug seeking. These screens are uncorrelated and implicated in separate drug abuse models. 50 kHz USVs measure affective state in rats and are evoked by positive stimuli. NSS and USVs are each implicated in drug response, self-administration, and reveal differences in individual behavior, yet their relationship with each other is not understood. The present study screened rats for their response to novelty and measured USVs of all call types in response to heterospecific play to determine the relationships between these individual difference traits. Generally, we hypothesized that 50 kHz USVs would be positively correlated with the NPP screen, and that 22 kHz would be positively correlated with the IEN screen. Results indicate none of the screens were correlated indicating they are measuring different individual difference traits. However, examination of the subtypes of USVs indicated harmonic USVs and the novelty place preference were positively correlated. Harmonic 50 kHz USVs increased in response to reward associated context, suggesting animals conditioned to the heterospecific tickle arena and anticipated rewarding stimuli, while FM only increased in response to tickling. USV subtypes can be used to elucidate differences in attribution of incentive value across conditioned stimuli and receipt of rewarding stimuli. These data provide strong support that harmonic and FM USVs can be used to understand reward processing in addition to NSS.

1. Introduction

Humans, rodents and other mammalian species have a tendency to seek out and explore novel environments and stimuli. In humans, the tendency to be a high novelty and sensation seeker is correlated with a variety of maladaptive behaviors such as unprotected sex and drug experimentation [1–3]. Therefore, several animal models have been developed to study the relationship between novelty and sensation-seeking (NSS) behavior and maladaptive behavior in rodents. When rodents encounter novel stimuli they show an increase in locomotor activity that ceases when novel stimuli become familiar or well explored [4]. Research suggests exploration of novel stimuli activates regions of the mesolimbic dopamine pathway, indicating the presentation of a novel stimulus or the exploration of a novel stimulus is rewarding to the organism [5,6]. Presentation of novel stimuli including odors, objects, visual or tactile cues can decrease amphetamine self-administration [7,8].

The inescapable novelty screen and novelty place preference screen are behavioral measures used to measure the response to novelty in rodents. The inescapable novelty screen challenges animals with a novel open-field apparatus and locomotor activity is measured [4]. Dopamine is thought to mediate the increase in locomotor response because inactivation of dopamine in the

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nucleus accumbens reduces novelty-induced locomotor response [9,10]. The 30 min inescapable novelty screen is sufficient to reveal differences in novelty-induced locomotor activity between rodents, which are subsequently divided into high responders (HR) or low responders (LR). High responding rodents display a variety of behaviors in which they are different than their lower responding counterparts including elevated locomotor response to low doses of psychomotor stimulants [11,12], and a faster rate of acquisition of self-administration of low unit doses of psychostimulants [4,7,13,14]. Interestingly, pharmacological interventions resulting in the elevation of dopamine in the mesolimbic pathway result in increased exploratory locomotion providing evidence that dopaminergic transmission mediates the locomotor response to a novel environment and to amphetamine-induced locomotor activity [9,10].

Similar to the inescapable novelty screen, the novelty place preference screen is used to measure the response to novelty in rodents. However, unlike the inescapable novelty screen where the novel environment is unavoidable, the novelty place preference screen measures the animal's choice to engage a novel context. In the novelty place preference screen animals can freely move between a familiar and novel context. Animals with greater amounts of time in the novel context are classified as high novelty seekers (HNP), and these HNP animals show a propensity to develop compulsive cocaine self-administration behavior, while low novelty seekers (LNP) do not show the same compulsive behaviors [15]. Similar to novelty-induced locomotor activity, elevated dopaminergic activity has been recorded in the mesolimbic pathway when animals engage the novel context [16]. This elevation in dopamine in this reward pathway is transient and is not induced by subsequent entries into the previously novel context, indicating the novelty aspect and not the contextual elements were responsible for the elevation in dopamine.

Despite the novelty screens' relationships with dopamine and addiction, they are uncorrelated, indicating the inescapable novelty screen and the novelty place preference screen are measuring different aspects of novelty [14,15,17]. One proposed hypothesis for the observed differences is that the inescapable novelty screen induces a stress response as evidenced by an elevation in corticosterone [13], while the novelty place preference screen does not elevate corticosterone [18]. Further support that these screens are measuring different aspects of novelty is that HR rodents as classified in the inescapable novelty screen are not necessarily HNP rodents in the novelty place preference screen, with similar results with LR rodents and LNP rodents [15]. Although each novelty behavior is mediated by dopaminergic activity, it is unclear how these screens correlate with other measures of individual differences. Understanding this relationship will bridge the gap between the NSS screens and elucidate novelty's complex relationship with addiction-like criteria and reveal how novelty is implicated in the processing of rewards [14,15].

Given that NSS in rats is complex and may encompass more than one dimension, another measure to understand the affective or motivational response of the rodent in real-time could be beneficial. Ultrasonic vocalizations (USVs) are emitted by rats and mice and are reflective of the animals' motivational and/or affective state [19,20]. Broadly, USVs can be dichotomized into 2 call types reflecting positive and negative affective states. Twenty-two kHz calls serve as alarm calls and are evoked by aversive stimuli including startling air puffs and withdrawal from drugs of abuse [21–23]. These calls are indicative of a negative affective state. Alternatively, 50 kHz calls are evoked by unconditioned rewarding stimuli including psychomotor stimulants [24–31], and tickling, termed 'heterospecific play', or anticipation of rewards [32–35].

Tickling is a procedure designed to mimic rodent play behavior and is rewarding to rodents. Rats will readily self-administer tickling and not general hand contact [36], and rats readily develop a conditioned place preference for a context paired with tickling [36]. 50 kHz vocalizations can be used as a measure of the individual incentive value attributed to the reinforcer, with greater numbers of 50 kHz vocalization indicative of a larger perceived magnitude of the reinforcer. Rodents with greater 50 kHz vocalizations demonstrated a greater conditioned place preference [37–39], indicating the subjective value of the reinforcer can be measured with 50 kHz vocalizations. Pharmacological manipulations of dopamine D_1 and D_2 receptor function suggest 50 kHz are strongly dependent on dopamine function [40]. Therefore; USVs could be used to understand the affective state of the rat to elucidate the different motivational states of NSS including the aversive and the rewarding elements.

Novel stimuli are thought to be rewarding, yet animal models of novelty are uncorrelated. Furthermore, the inescapable novelty screen is known to elevate corticosterone and may be a measure of stress-induced locomotor activity rather than novelty-induced locomotor activity, while the novelty place preference screen measures the animal's choice to engage a novel context. The present study examined rat behavioral responses in the inescapable novelty screen, novelty place preference screen, and to heterospecific play, to determine the relationship between NSS and USVs. We hypothesized the inescapable novelty screen and 22 kHz vocalizations would be positively correlated because the inescapable novelty screen induces a stress response and aversive stimuli or negative affective states result in 22 kHz vocalizations. In addition, we hypothesized the novelty place preference and 50 kHz vocalizations would be positively correlated, because animals that engage the novel context find the novel context more rewarding and therefore will vocalize more at 50 kHz. Our results indicate that globally, the individual difference screens were not correlated, suggesting they were each measuring a different individual difference trait and that novel behavior in the rat is complex. However, harmonic and frequency modulated (FM) changed differently with repeated tickling and demonstrate that subtypes of 50 kHz USVs can be used to understand attribution of incentive value associated with reward. Significant relationships were revealed between the novelty place preference screen and the change in harmonic 50 kHz USVs measured during different phases of the experiment. Together these results indicate that subtypes of 50 kHz USVs may be capable of elucidating affective/motivational differences observed in the novelty place preference screen and attribution of incentive value.

2. Methods

2.1. Animals

Fifty 30-day-old male, Sprague-Dawley rats were obtained from Charles River Laboratories. All animals were housed in a temperature and humidity controlled colony room. Animals were housed individually in transparent polyurethane cages with Carefresh rodent bedding with access to food and water ad libitum. Upon arrival all animals were handled, but not tickled, for ~1 min daily to facilitate experiment handling procedures. Experimentation began 10 days after arrival. The colony room was maintained on a 12:12 light–dark cycle, with lights on from 7:00 to 19:00. All behavioral testing occurred in the light cycle. Based on previous literature, animals were tested in the light cycle [17,36,41]. All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee and complied with the Guide for the Care and Use of Animals (National Research Council, 2011).