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The possible effects of isolation and conspecifics in the emission of 22kHz USVs in adult rats

Studies have reported that rats emit ultrasonic vocalizations (USVs) in a number of biologically significant situations (Blanchard, Blanchard, Agullana, & Weiss, 1991; D'Amato, Scalera, Sarli, & Moles, 2005; Van der Poel & Miczek, 1991). Early in development, rat pups have been known to emit USVs during separation from dams (Winslow & Insel, 2002) and littermates (Hofer, 1994). The USVs emitted in these situations have been considered a measure of separation anxiety and discomfort. It has also been suggested that the 22 kHz USVs are good measure of fear in laboratory rats (Blanchard et al., 1991; Brudzynksi & Chiu, 1995). Additionally, 22 kHz USVs have been used to measure general distress induced by nociceptive stimuli (Van der Poel & Miczek, 1991), such as acetic acid injections (Portavella, Depaulis, & Vergnes, 1993). Therefore, USVs, specifically 22 kHz USVs in adult rats, are considered a good indicator of emotional status in aversive circumstances and can be induced by nociceptive stimuli.

In addition to providing the emotional status of rats, it has been suggested that USVs also serve as form of intraspecific communication, which is dependent on the presence or absence of a conspecific. D'Amato and Populin (1987) have shown that normal hearing pups in social isolation conditions significantly decrease the emission of USVs when crossed fostered with deaf dams. D'Amato and Populin (1987) interpreted the latency of deaf dams to respond to pups' calls as a factor that was responsible in reducing USVs. Thus, it seems likely that the pups know when the emission of USVs are effective in eliciting maternal care and emit more USVs in the presence of non-deaf dams. Similarly, in adult rats, the emission of 22 kHz USVs during and after exposure to a predator is greatly facilitated by the presence of an audience, that audience being familiar conspecifics (Blanchard et al., 1991). Additionally, during aggressive interactions between male adult rats, 22 kHz USVs have been recorded from subordinate rats, which are thought to be an indication of submission in order to inhibit attacks from dominant rats (Sales,

1972). Rats emit USVs early in development in order to communicate with dams and this behavior is also seen in adulthood, serving the same purpose of communication. It is likely that rats learn to emit USVs in biologically significant situations due to the presence and responses of conspecifics. It has already been shown that rat pups reared in complete isolation through the process of artificial rearing (AR) have been shown to display abnormal ongoing social behavior and social learning (Levy, Melo, Galef, Madden, & Fleming, 2003), e.g. impairment of maternal behavior and social learning of food preference in adulthood (Melo, Lovic, Gonzalez, Madden, Sinopoli, & Fleming, 2006). Therefore, environmental conditions pertaining to the presence or absence of conspecifics early in life should affect the emission of USVs in adulthood.

The purpose of this study is to investigate the possible effects that social isolation through artificial rearing methods may have on the emission of acetic acid-induced 22 kHz USVs in adult rats. It is of interest to see whether the presence of a conspecific during the induction of the aversive stimulus (acetic acid injection) will affect the amount of 22 kHz USVs emission in both isolated and non-isolated groups. It is expected that rats reared artificially and kept in complete isolation will emit less 22 kHz USVs than non-isolated rats when USVs are induced by an IP injection of 3.2% of acetic acid and during the presence of a conspecific. In general, the study will help provide a better understanding on how the absence or presence of a conspecific may serve as a mechanism in eliciting USVs and how the same mechanism shapes the development of USVs.

Methods

Design/Subjects

Thirty male Sprague-Dawley rats will be used as test subjects. The study will consist of 2 experimental groups and testing will begin at 80 days of age. The 2 experimental groups will be

identified as isolated and non-isolated. The isolated group will consist of fifteen rats reared and kept in complete isolation from birth using a paradigm of artificial rearing (Patel, Satyaprasad, & Johanning, 1994). The non-isolated group will consist of fifteen rats that will be socially reared from birth and will be housed in groups of 3 prior to testing.

Dependent variables are the amount of 22 kHz USVs emissions. Independent variables are differences in rearing environment (isolated and non-isolated), 3.2 % of acetic acid injection, and presence or absence of a conspecific.

Apparatus:

All tests will take place in a silent room. A transparent observational box with 2 separate identical chambers divided by a mesh screen will be used during testing. One chamber will be designated for test subjects.

USVs will be induced in test subjects by an IP injection of 3.2% of acetic acid. USVs will be detected using a bat detector tuned to 22 kHz. The microphone of the bat detector will be placed above the testing chamber that will contain the test subject.

The conspecific will be an adult male Sprague-Dawley rat with a cut larynx. The cut larynx will inhibit any possible USV emission that could interfere with the recording of USVs emission from test subjects.

Procedure

The experiments will be conducted for 10 consecutive days and done randomly in order to prevent an order effect. All test subjects will be tested 5 times each in the presence and absence of a conspecific. The number of individual USVs emitted will be recorded in both contexts.

In the presence of a conspecific, test subjects will be given IP injections of 3.2% of acetic acid and placed immediately into the chamber designated for test subjects with the presence of a conspecific in the other chamber. Both the conspecific and the test subject will be placed close to the mesh screen in the beginning of the test, so test subjects will be aware of the conspecific. Five minutes after injection, recordings of 22 kHz USVs will take place for 30 minutes.

In the absence of a conspecific will be given IP injections of 3.2% of acetic acid and placed immediately into the chamber designated for test subjects. Five minutes after injection, recordings of 22 kHz USVs will take place for 30 minutes.

Anticipated Results

It is anticipated that rats in the isolated group will produce the same amount of 22 kHz USVs during the presence and absence of a conspecific. Non-isolated rats are expected to emit more 22 kHz USVs during the presence of the conspecific stimulus than absence. Additionally, it is anticipated that non-isolated rats will emit more 22kHz USVs in both experimental tests than isolated rats. However, it is probable that an increase of 22 kHz USVs may occur in both isolated and non-isolated groups with repetitive exposure to a conspecific.

Discussion

It has been established that several developmental and environmental manipulations have an important impact on the behavior of rats. For instance, isolation has been known to cause deficits in the establishment of normal rat behaviors (Levy et al., 2003; Melo et al., 2006). Normal behaviors may include the emission of USVs in intraspecific communication. Ingaki, Kuhwahara, Kikusui, and Tsubone (2005) have shown that singly reared rats after weaning emitted fewer stress-induced 22 kHz USVs (by acute mild somatic stimuli) than socially reared rats. According to Ingaki et. al, (2005), these results revealed that the lack of social interaction

after weaning decreased the emission of 22 kHz USVs and that 22 kHz USVs in rats are developed through social interaction after weaning. Furthermore, it has been observed that in aversive situations, the emission of 22 kHz USVs by adult rats is facilitated by the presence of familiar conspecifics (Blanchard et al., 1991). Thus, it is hypothesized that social isolation through artificial rearing will cause deficits in the emission of 22 kHz USVs in adult rats when exposed to aversive stimuli (IP injection of 3.2% of acetic acid) in adulthood.

It is also probable that isolated rats may emit the same amount of USVs as non-isolated or even more. A study by Tomazini, Reimer, Albrechet-Souza, and Brandao (2005) showed that adolescent rats (40 days old) isolated for 1 day showed a significant increase in the number and duration of USVs compared to non-isolated groups when exposed to unconditioned aversive stimuli. However, the same study showed that rats isolated for two-weeks emitted less USVs than non-isolated group. Rosa, Nobre, Oliveira, and Brandao (2005) found that rats isolated for 10 days after weaning emitted less ultrasonic vocalizations than grouped animals when exposed to a novel environment. Furthermore, resocialization was not able to counteract the effects of isolation, which suggests that the emotional state of the animals is altered by 10 days of isolation. Therefore, it is likely that the effects isolation has on USVs is age-dependent and length dependent, where exposure to isolation for longer periods of time at earlier ages in rats causes greater deficits in USVs emission than short exposures to isolation at later ages in rats.

Accordingly, the age when rats are isolated and the age of exposure to conspecifics can affect the production of USVs in rats. This study aims to see whether the effects of isolation will carry into adulthood and whether repetitive conspecific exposure can reverse the effects of isolation. If the effects of isolation can be reversed then future research could determine the underlying neural process that mediate these changes in order to better understand the effects of

isolation on the brain. Thus, by conducting this study we can gain insight about learning in general and the variables that affect learning during development.

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