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Prenatal exposure to vanilla or alcohol induces crawling after these odors in the neonate rat: The role of mu and kappa opioid receptor systems

Mirari Gaztañaga, P. Ezequiel Aranda-Fernández, M. Gabriela Chotro *

Faculty of Psychology, University of the Basque Country UPV/EHU, Avda. de Tolosa, 70, 20018 San Sebastián, Spain

HIGHLIGHTS

- Newborns exposed prenatally to vanilla or alcohol crawl attracted by these odors
- Prenatal Naloxonazine with alcohol eliminated neonate attraction for alcohol odor
- Prenatal Naloxonazine with vanilla eliminated neonate attraction for vanilla odor
- Prenatal nor-Binaltorphimine with alcohol reduced neonate attraction to alcohol odor.
- Prenatal nor-Binaltorphimine with vanilla did not affect neonate attraction to vanilla odor.

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ABSTRACT

Rat fetuses can perceive chemosensory stimuli derived from their mother's diet, and they may learn about those stimuli. In previous studies we have observed that prenatal exposure to alcohol during the last days of gestation increases the acceptance and liking of an alcohol flavor in infant and adolescent rats. While these results were not found after prenatal exposure to vanilla, cineole or anise, suggesting that the pharmacological properties of alcohol, mediated by the opioid system, underlie the effects observed with this drug. Considering that other studies report enhanced acceptance of non-alcohol flavors experienced prenatally when subjects were tested before infancy, we explore the possibility of observing similar results if testing 1-day old rats exposed prenatally to vanilla. Using an "odor-induced crawling" testing procedure, it was observed that neonates exposed prenatally to vanilla or alcohol crawl for a longer distance towards the experienced odor than to other odors or than control pups. Blocking mu, but not kappa opioid receptors, reduced the attraction of vanilla odor to neonates exposed to vanilla in utero, while the response to alcohol in pups exposed prenatally to this drug was affected by both antagonists. Results confirm that exposure to a non-alcohol odor enhances postnatal responses to it, observable soon after birth, while also suggesting that the mu opioid receptor system plays an important role in generating this effect. The results also imply that with alcohol exposure, the prenatal opioid system is wholly involved, which could explain the longer retention of the enhanced attraction to alcohol following prenatal experience with the drug.

It is well acknowledged that the near-term rat fetus has the capacity to perceive chemosensory stimuli presented in its prenatal environment [31,32,37,42] and that it is able to learn about those stimuli, showing habituation and sensitization to them [12,30,43], as well as Pavlovian associative learning [46]. Fetuses can be exposed to chemosensory stimuli in their amniotic environment indirectly, derived from the mother's diet, and learning may also occur about those stimuli. Some studies have found that maternal ingestion of flavored substances increases the offspring's postnatal acceptance of that flavor (odor and taste). For example, in humans it has been found that newborns express a preference for anise odor [40], or less aversion to the odor of garlic [21] when their mothers had consumed those substances during the last period of pregnancy. It has also been reported that six-month old babies express preference for carrot-flavored food when their mothers had consumed daily amounts of carrot juice during the last 3 weeks of pregnancy [29].

Preferences for chemical stimuli included in the maternal diet during gestation have also been reported in several species of mammals (for a review see [39]). However, in only a few of those studies was exposure restricted to the prenatal period, while in most of them the sensory experience continued to peri- or postnatal stages, by maintaining the administration of the stimulus until the onset of parturition, or even explicitly re-exposing the neonates or the mothers to the tastant during lactation. In those cases, it is highly probable that the increased liking for the tastant was induced by perinatal and/or postnatal appetitive associations of the stimuli (perceived either in amniotic fluid, during birth, or in milk or saliva during lactation) with the reinforcing consequences

* Corresponding author.
E-mail address: g.chotro@ehu.es (M.G. Chotro).

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of maternal interaction, such as physical stimulation, suckling, and lactation [35]. In the few studies in which subjects were exposed to a chemosensory stimulus exclusively during the prenatal period, without explicit contiguity with a reinforcer, the results appear to be mixed. Some studies have reported no preference for those tastes or odors, while others have found an effect of increased preference for the preexposed stimuli [15]. In sum, the results of the majority of these studies with rats do not consistently support the idea of a preference established exclusively by mere prenatal exposure to chemosensory stimuli.

With prenatal exposure to alcohol (ethanol) the literature shows quite different results. In most studies with rats in which alcohol was administered to the pregnant rat during gestation, the offspring consistently show an increased acceptance of alcohol during infancy, adolescence and even in adulthood [10]. In addition to its pharmacological effects, alcohol has a distinctive flavor (i.e. the integration of gustatory, olfactory, and trigeminal or irritant components), and is one of those substances from the maternal diet that reaches the fetus and the amniotic fluid. When the pregnant mother consumes alcohol, this relatively small molecule passes directly from the mother through the placenta, reaching the fetal blood at similar levels to those found in maternal plasma [48]. From fetal circulation, alcohol is eliminated mainly through the maternal metabolism [18], and it accumulates in the amniotic fluid, reaching higher levels than in the maternal blood and taking longer to be eliminated [20]. Hence, after maternal alcohol ingestion, the fetus is exposed to the drug's pharmacological effects as well as to its chemosensory properties. Many studies with rodents have demonstrated that alcohol exposure during the entire gestation of the rat induces increased intake of alcohol after birth [10]. This effect has also been reliably found when the drug is administered exclusively during the last days of pregnancy, on gestational days (GD) 17–20 (for example, [9,11,16]). It was also shown that the effect of increased alcohol intake is accompanied by an enhanced palatability of the flavor of alcohol [3,4]. In addition, it is known that on GD 17 the fetal opioid system of the rat is already functional, particularly the mu and kappa opioid receptor systems, while the delta opioid receptor system develops after postnatal day 14 [13]. Also, that administering the pregnant rat with the non-selective opioid antagonist naloxone or naltrexone, together with alcohol, prevents the increased alcohol acceptance. Taken together, these findings clearly suggest that the effect is mediated mainly by the endogenous opioid system [3,9,11,52]. We were thus able to conclude that after maternal alcohol ingestion, the rat fetus learns a conditioned response to the chemosensory properties of alcohol, associating them with an appetitive reinforcer whose effects are mediated by the endogenous opioid system. The identity of the reinforcer has recently been investigated, examining the role of the two main candidates; one is alcohol itself, given that alcohol induces the release of opioids [17,22] and that its reinforcing effects are mediated mainly through the stimulation of the delta and mu opioid receptor systems [2,22,47]. The other candidate is the amniotic fluid via one of its components, known as the kappa-inducing factor (KIF) [39], which has been found to induce the stimulation of the kappa opioid receptor subsystem, when it enters the subject's mouth [25,26,39]. It has been suggested that the activity of KIF from GD 20 may be the agent that mediates prenatal conditioned preferences to flavors, including the effects of an increased liking for alcohol observed in our studies. This last possibility is consistent with results showing that a prenatal alcohol exposure effect was observed in infant and juvenile rats when exposure was on GD 19–20, but not on the previous days [14]. Nonetheless, and contrary to our expectations on the basis of the evidence previously described, we have found that the administration of anise or vanilla during those same days of gestation (19–20) did not induce increased intake and enhanced palatability of these flavors when tested in infancy, unlike the case with alcohol [15]. Furthermore, in the same study it was found that blocking the fetal mu opioid receptors completely annulled the effects of enhanced intake and palatability of alcohol after prenatal exposure to the drug, while antagonizing kappa opioid receptors only partially reduced the effects on palatability. This latter evidence implies that the main appetitive reinforcer involved in the prenatally conditioned preference to alcohol detected two weeks after birth was the pharmacological effect of alcohol on the fetal mu opioid system, although both opioid receptor systems appear to be involved.

To summarize, in our previous studies with prenatal exposure to non-alcohol flavors we did not find an increased acceptance of those flavors when measured on postnatal day (PD) 14, whereas with prenatal alcohol we did find such an effect. Given our own findings and the inconsistent evidence concerning the effects of prenatal exposure to a non-alcohol flavor [15], it is clear that there is an important difference in the effects produced by prenatal exposure either to alcohol or to other flavors lacking pharmacological effects. Nevertheless, the nonappearance of an enhanced acceptance of vanilla or anise, when tested two weeks after birth, does not necessarily prove the absence of a prenatally acquired appetitive response. It remains possible that prenatal learning is occurring (associative or non-associative) but that it generates a weaker memory, which is not retained until infancy, but could be detected soon after birth. It is well acknowledged that there are differences in retention and forgetting in subjects of different levels of maturity, i.e. young rats show more rapid forgetting than older ones [45]. Therefore, using newborn rats the aim of the present study was to test this hypothesis by evaluating the attractiveness of an odor to which they were exposed prenatally.

In spite of the relatively limited behavioral repertoire of the newborn rat (PD 0–1), there are several examples of tests designed to evaluate the preference or acceptance of flavors in this population. Such tests include, for instance, the counting of mouth movements and licks after oral-lavage with a tastant (e.g. [31,32]) or after intraoral infusions of the flavors through a cheek-implanted cannula (e.g. [1]), operant conditioning reinforced by a flavored substance infused through an intraoral cannula (e.g. [23,33]); conditioning of an artificial nipple through which the flavor was delivered [38]; odor conditioning with the artificial nipple procedure [8]; the attachment of a pup to the odor-scented nipples of anesthetized females (e.g. [36]); and most recently a test of "odor-induced crawling locomotion" in newborn rats [27]. Among all of these tests, the latter has been proven successful for assessing the attractiveness of odors in rat neonates. In this study, with a comparatively stress-free preparation for the newborn rat, unbiased patterns of response towards naturally attracting odors, milk or amniotic fluid were demonstrated, while pups clearly displayed almost no response to the presentation of other substances such as anise or water [27].

On the basis of this information, we decided to use this test to evaluate changes in the attractiveness of the odors of vanilla or alcohol in 1-day old pups exposed prenatally to these substances on GD 17–20 (Experiment 1). Using this same testing procedure we also evaluated the neonatal response after blocking mu and kappa opioid receptors during the prenatal exposure to either vanilla (Experiment 2) or alcohol (Experiment 3).

1. General methods

1.1. Subjects

Subjects were a total of 306 one-day old Sprague–Dawley rat pups, derived from 51 litters, born and reared in a temperature-controlled vivarium at the Department of Psychology, University of the Basque Country, SPAIN. The colony room was maintained on a 12-h light/12-h dark illumination cycle, with light onset at 8:00 am. Female and male Sprague Dawley adult rats (Vivarium UPV/EHU, Leioa, Spain) were time-mated to provide subjects for this study, and the presence of sperm in vaginal smear was considered as GD 0. Pregnant females were housed in pairs in standard maternity cages with continuous access to food and filtered water, and remained undisturbed until the