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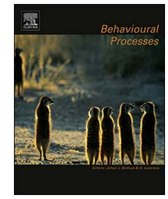

# Successive odor matching- and non-matching-to-sample in rats: A reversal design

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## ABSTRACT

There is a growing body of research on matching- and non-matching-to-sample (MTS, NMTS) relations with rats using olfactory stimuli; however, the specific characteristics of this relational control are unclear. In the current study we examine MTS and NMTS in rats with an automated olfactometer using a successive (go, no-go) procedure. Ten rats were trained to either match- or non-match-to-sample with common scents (apple, cinnamon, etc.) as olfactory stimuli. After matching or non-matching training with four odorants, rats were tested for transfer twice with four new odorants on each test. Most rats trained on MTS showed immediate transfer to new stimuli, and most rats trained on NMTS showed full transfer by the second set of new odors. After meeting criterion on the second transfer test, the contingencies were reversed with four new odor stimuli such that subjects trained on matching were shifted to non-matching and vice versa. Following these reversed contingencies, the effects of the original training persisted for many trials with new odorants. These data extend previous studies on same-different concept formation in rats, showing strong generalization requiring few exemplars. The critical role of olfactory stimuli is discussed.

## 1. Introduction

As identity and oddity are two of the most elemental concepts of learning, they have been the focus of most recent research on concept learning in nonhumans. Identity and oddity can be operationalized by same/different or match-/non-match-to-sample (MTS/NMTS) procedures, such that successful transfer to novel stimuli defines the emergence of concept learning. Using such procedures, identity/oddity has been demonstrated in a number of species, including primates (D'Amato et al., 1985; Katz et al., 2002; Vonk, 2003), dolphins (Herman et al., 1989), sea lions (Kastak and Schusterman, 1994), harbor seals (Scholtyssek et al., 2013); echidna (Russell and Burke, 2016), pigeons and other birds (Bodily et al., 2008; Magnotti et al., 2015; Wright et al., 1988), and honeybees (Giurfa et al., 2001). Initial studies with rodents using visual stimuli (e.g., Iversen, 1993; Iversen, 1997) failed to show identity/oddity but with the use of olfactory stimuli there has been more success (e.g., April et al., 2011; Lu et al., 1993; Otto and Eichenbaum, 1992; Peña et al., 2006; Prichard et al., 2015).

For example, using an olfactory discrimination procedure, Peña et al. (2006) trained rats to dig in sand scented with common household spices to obtain sucrose pellets (cf. Dusek and Eichenbaum, 1997) and found evidence for generalized matching-to-sample. Rats were initially trained on a single conditional discrimination (two olfactory stimuli) and novel olfactory stimuli were added as criterion level performances were reached. At the end of the study, rats were matching at high levels of accuracy with 20 or more different stimuli and responses to novel stimuli were well above chance levels in three of the four rats tested. However, as novel stimuli were only introduced one or two at a time, it was not possible to identify precisely at what point generalized matching developed.

April et al. (2011) used a similar olfactory discrimination procedure to train six rats on either MTS or NMTS. In this study, a reversal procedure based on the Zentall and Hogan (1974) study with pigeons was used such that after initial MTS or NMTS training, contingencies were switched and transfer assessed. Zentall and Hogan inferred concept learning from response persistence to the originally trained contingency. April et al. trained rats on either MTS or NMTS with five scent stimuli (set A); once rats responded with 90% accuracy, they were switched to five new stimuli (set B) and showed evidence of savings in these transfer tests. After 15 sessions with these stimuli (set B), a new stimulus set of five odors (set C) was presented with the previous contingencies reversed. Initial levels of accuracy were quite low as all animals continued to respond in line with the original MTS or NMTS contingency from sets A and B. Even after extended training, most

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animals failed to exceed chance levels of responding on the new contingency with set C. This study showed that rats developed the identity or oddity relation with as few as 10 exemplars.

The studies noted above used scented sand to present the odorants and a manual procedure with simultaneous conditional discrimination training. In the Peña et al. (2006) study, rats were tested in an operant chamber modified to allow the experimenter to manually insert a tray with the sample and the two comparisons. In the April et al. (2011) study, rats were presented with sample stimuli in a holding cage, then moved to a circular arena in which the comparison stimuli were presented. An alternative to this approach is to use automated presentation of the odorants in an operant chamber and record nose-poking behavior at the port where the scent is introduced. Instead of a two-alternative choice procedure for presentation of comparison stimuli, a go, no-go procedure is trained. After the sample odor is presented, one comparison odor is presented, and the rat learns to "go" (nose-poke) to earn reinforcement when the comparison matches the sample (in a MTS paradigm) and to withhold responding (no-go) when the comparison does not match the sample. The automated procedure increases experimental control by minimizing effects of handling and other distractions for the subject, as well as providing a more precise dependent measure. Slotnick and colleagues (see Slotnick, 2001 for a review) developed this procedure for rats and mice, demonstrating both discrimination and MTS with odor stimuli. In particular, Lu et al. (1993) used an automated procedure to test odor matching in rats, using successive conditional discrimination training (go, no-go). They found that rats learned an olfactory MTS task even with delays with a masking odor up to 10 s between stimuli and showed rapid transfer of learning to new sets of odor stimuli. However, because performance on initial transfer tests (before reinforcement) was not presented, it was not possible to determine whether transfer involved generalized control by the identity relation or by rapid learning of new stimulus sets.

Using a similar automated procedure, Prichard et al. (2015) trained six rats on a go, no-go MTS procedure with four odor stimuli. Stimuli were presented in pairs and nose-poke responses to matching odor pairs, but not non-matching pairs, were reinforced. Once rats met criterion responding, non-reinforced probe trials with novel odors were intermittently introduced. Most rats showed high levels of transfer, suggesting that four exemplars (i.e., four different odors and eight trial-type combinations) may be sufficient for emergence of the identity relation. This outcome was surprising as most studies have found that many more trained exemplars are necessary to produce reliable transfer in other species (Katz and Wright, 2006; Wright et al., 2016).

In the current study, we were interested in extending the research of Prichard et al. (2015) to include an analysis of the non-identity as well as the identity relation. Further, we wanted to examine transfer across stimuli and persistence of the original contingencies to infer concept learning. Thus, we used the same automated olfactometer set-up to present odor stimuli with a successive discrimination procedure to replicate and extend Prichard et al. (2015), and employed a reversal design, similar to Zentall and Hogan (1974) and April et al. (2011).

## 2. Method

### 2.1. Subjects

The subjects of this experiment were 15 male Sprague-Dawley albino rats approximately 90–150 days old at the beginning of training. Some of the animals were trained to lever-press prior to beginning the present study, but all were naïve to training with odor stimuli and the olfactometer procedures. All rats were individually housed on a reversed 12-h light-dark cycle. The rats were maintained at 85 percent of their free feeding weight and received *ad libitum* access to water in their home cages. All experiments were performed during the dark phase, between 7:00 a.m. and 7:00 p.m. Rats were fed Lab Diet Rat Chow daily approximately 1 h following their individual experimental session. Animals were maintained and data were collected in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*; all researchers completed IACUC training and the study was approved by the UNCW Animal Care and Use Committee.

### 2.2. Apparatus

Sessions were conducted in Med Associates operant chambers with three response ports located across the front panel; however, only the center port (2.5 cm diameter) was activated during the experiment. Inside the center port was a stimulus light, infrared photo beam response detector, and openings for scents to be pumped in and drawn out. The chamber measured 30.5 cm long by 24 cm wide by 21 cm high with a pellet dispenser located on the opposite side of the chamber from the response ports. Chambers were housed in sound attenuating cubicles. Each chamber was interfaced to a computer equipped with MED-PC software. Three five-channel Med Associates olfactometer systems (ENV-275-5) were added to each chamber. An input pump (Linear AC0102, 2.84 pound per square inch with an airflow of .177 cubic feet per min) delivered air through glass jars containing an odorant solution to solenoids that, when operated, forced scented air through Teflon tubing and a manifold into the center nose port of the chamber. A vacuum pump (Linear VP0125, −9.84 Hg vacuum and air displacement of .247 cubic feet/min) removed air from a tube located at the bottom of the center port. Thus, the system was capable of delivering 15 separate odors through the center response port [see Prichard et al. (2015) for an illustration].

### 2.3. Odorants

Liquid odorants purchased from The Great American Spice Company, Nature's Garden, and local stores were used to create four sets of stimuli: Set A (cinnamon, apricot, bubblegum, root beer), Set B (brandy, vanilla butternut, almond, licorice), Set C (apple, grass, coconut, sandalwood), Set D (clove, honey, blueberry, geraniol). A fifth set (E: lemon, maple, lavender, peppermint) was used with one rat but the peppermint oil appeared to contaminate the apparatus and disrupt performance, so these scents were discontinued. Odorants were diluted to a solution of 6.7 ml oil per 100 ml distilled water. Glassware was cleaned at the end of each testing day and solutions were refilled every morning.

### 2.4. Procedure

#### 2.4.1. Shaping phase

An initial session of magazine training was followed by response training in which both the center port light and house light were illuminated. During this phase, a single nose-poke turned off both lights, and delivered a sugar pellet accompanied by a light above the food hopper. After a 5-s period, the hopper light went out and the house and center-port lights came on and the procedure continued to provide reinforcement on a FR1 schedule. Once regular responding was established, the reinforcement schedule was progressively increased to FI–5 s over several sessions. To acclimate animals to scent delivery through the center port, four odorants were introduced for each rat (see Table 1). Each trial began with the onset of the house and center port lights and delivery of one of the four odorants; completion of the FI–5 s schedule terminated the lights and odorant delivery and produced reinforcement and the onset of the hopper light for 5 s.

#### 2.4.2. Initial conditional discrimination training phase

Once rats were consistently responding to all four scents throughout the session, conditional discrimination training began. Rats were randomly assigned to either MTS or NMTS training and began training with the initial stimulus set used in shaping (see Table 1). All trials consisted of stimulus pairs presented through the center port, and only