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## Supporting Online Material for

### **Causal Reasoning in Rats**

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Materials and Methods  
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## Supporting Online Material

### Experiment 1

*Subjects.* Thirty-two experimentally-naïve, male Long-Evens rats (*Rattus norvegicus*) were pair-housed in plastic tubs with wood shaving substrate in a vivarium maintained on a 12-hr dark/light cycle. Experimental manipulations occurred during the dark portion of the cycle. Rats were maintained at 85% of free-feeding weights and were handled prior to the study. Rats were randomly assigned to two of four conditions ( $ns = 16$  per condition), either Intervene-T and Observe-N, or Observe-T and Intervene-N.

*Apparatus.* Each of eight identical experimental chambers measuring 30 L x 25 W x 20 H cm was housed in a separate sound- and light-attenuating chest. The walls and ceiling of the chamber were constructed of clear Plexiglas and the floors were constructed of stainless-steel rods measuring 0.5 cm in diameter, spaced 1.5 cm center-to-center. The enclosure also contained a 28-V, 100 mA shielded incandescent house light and a diffuse light mounted on the left-side wall of the conditioning chamber.

Each chamber was equipped with a liquid dipper that could deliver .05 cc sucrose solution (20%). Three speakers on the outside walls of the chamber could deliver a high-frequency tone stimulus (3000 Hz) 8 dB(A) above a background noise of 62 dB(A), a white noise stimulus 8 dB(A), and a click train stimulus (6/s) 8 dB(A). A flashing light (2/s) could be produced by turning off the house light and flashing the diffuse light. All stimuli, including sucrose delivery, were 10s in duration. Levers could be inserted into the cage 4 cm to the left of the food niche, 6.5 cm above the floor.

*Procedure.*

*Magazine training.* Day 1: The levers were retracted and 10s access to sucrose was delivered every  $20 \pm 15$ s to train rats to approach the feeding niche.

*Causal model training.* Days 2-7: All rats received four of each of the following daily trials pseudorandomly interspersed within each session with an interval of  $5 \pm 3$  min: Light or click (Stimulus L; counterbalanced within group) followed by Stimulus T (tone or noise, counterbalanced within group) (onset of T coincided with the termination of L); L followed by F (onset of F coincident with the termination of L); and Stimulus N (noise or tone, counterbalanced within group) and F simultaneously presented (with co-onsets and co-terminations). (We presented N and F simultaneously because, based on a common-cause model assumption, it would be rational to expect T and F to occur simultaneously.)

*Testing.* Levers were extended into the chambers for testing. Day 8: Rats in Condition Intervene-T received a 10s presentation of T each time they pressed the lever (except that lever presses during the presentation of T had no nominal consequence). Each lever press by a rat in Condition Intervene-T also caused the presentation of T for a rat in Condition Observe-T in an adjacent chamber (i.e., a yoking procedure). Rats in Condition Intervene-N received a presentation of N for each lever press, and caused the presentation of N for a rat in Condition Observe-N in an adjacent chamber. Day 9: Rats that received Condition Intervene-T on Day 8 received Condition Observe-N on Day 9, rats that received Condition Observe-T on Day 8 received Condition Intervene-N on the Day 9, rats that received Condition Intervene-N on Day 8 received Condition Observe-T on Day 9, and finally, rats that received Condition Observe-N on Day 8 received

Condition Intervene-T on Day 9. This counterbalancing scheme ensured that rats tested for lever pressing for one type of stimulus (e.g., T) on the first test session received yoked presentations of the other stimulus (e.g., N) on the second test session, and vice versa. Test sessions were 30 min. Lever pressing in the Observe conditions had no nominal consequence. All lever presses and nose pokes were recorded. In all experiments reported here, subjects nose poking more than two standard deviations below their group mean during the last session of acquisition were excluded from analysis for failing to acquire nose poke responding to Stimulus L. Data from five subjects (three from conditions Intervene-X/Observe-Y, and two from conditions Observe-X/Intervene-Y) that met this elimination criterion were discarded. Furthermore, data from four subjects (two from condition Intervene-X/Observe-Y, and two from condition Observe-X/Intervene-Y) were lost due to equipment failure, leaving a final  $n = 23$  for data analyses.

### *Results*

No differences among the test conditions were found in mean number of nose pokes during the background—in the absence of the test stimulus—indicating no contribution of baseline levels of responding to the test stimulus (Means  $\pm$  SEM =  $279 \pm 57$ ,  $305 \pm 99$ ,  $288 \pm 82$ , and  $284 \pm 49$  for Conditions Intervene-T, Observe-T, Intervene-N, and Observe-N, respectively). A two-way mixed ANOVA on training model (Common-Cause vs. Direct-Cause) and testing condition (Intervene vs. Observe) found neither main effects nor an interaction,  $F_s(1, 21) < 1.0$ .

A two-way mixed ANOVA on mean number of nose pokes (max = 100) to T or N at test found a main effect of causal model (Common-Cause vs. Direct-Cause),  $F(1, 21) =$

6.01,  $P < .05$ , and an interaction between causal model and testing condition (Intervene vs. Observe),  $F(1, 21) = 4.31$ ,  $P = .05$ . Planned comparisons revealed less nose poking in Condition Intervene-T than in Condition Observe-T,  $F(1, 21) = 5.12$ ,  $P < 0.05$ , while nose poking did not differ in Conditions Intervene-N and Observe-N,  $F(1, 21) < 1.0$ . Rats in Condition Intervene-T nose poked less than rats in Condition Intervene-N,  $F(1, 21) = 10.02$ ,  $P < .01$ , but there were no differences in mean nose pokes between Conditions Observe-T and Observe-N,  $F(1, 21) < 1.0$ . Thus, only interventions in effect T of a common cause attenuated nose poking at test.

Perhaps nose poking was lower in Condition Intervene-T than in Condition Observe-T simply because nose poking and lever pressing were incompatible responses. If a rat in Condition Intervene-T was lever pressing at the onset of T, it could not simultaneously place its nose in the feeder. There are two arguments against this possibility. First, nose-poke scores were high in Condition Intervene-N, which did not differ from Condition Observe-N. Thus, it was physically possible to first lever press and subsequently nose poke at a high rate. Second, no difference was found in the rate of lever pressing between Condition Intervene-T ( $M \pm SEM = 21 \pm 6$ ) and Observe-T ( $M \pm SEM = 19 \pm 6$ ),  $t(17) < 1$ . No difference in the rate of lever pressing was found for Conditions Intervene-N ( $M \pm SEM = 18 \pm 5$ ) and Observe-N ( $M \pm SEM = 11 \pm 4$ ),  $t(17) = 1.46$ ,  $P > .10$ , further denouncing the response interference explanation.

One might expect N to act as a conditioned reinforcer (S1), and thereby support a higher rate of lever pressing in Condition Intervene-N than in Observe-N. Although there was a non-significant tendency in this direction, our procedure was not designed to assess

conditioned reinforcement, which is parameter dependent and typically a relatively weak effect compared to primary reinforcement. A large number of first-order pairings (e.g., 120 (S2) or 600 (S3)) are typically required to produce conditioned reinforcement (our procedure had only 24 N-F pairings), and testing conventionally involves two levers (we only had one), one producing the conditioned reinforcer, the other producing a novel stimulus (S2-S3).

#### Experiment 2a

*Subjects and Apparatus.* Forty rats participated as in Experiment 1. Apparatus and stimuli as in Experiment 1, except that only the flashing light served as L and the tone and noise served as T and N, counterbalanced within group.

*Magazine training.* As in Experiment 1.

*Phase 1: Sensory preconditioning.* Days 2-5: Rats in Groups Common-Cause-Intervene and Common-Cause-Observe each received 6 daily trials of L→T (i.e., L followed by T) and N pseudorandomly interspersed. (N trials were included to reduce generalization). Rats in Groups Chain-Intervene and Chain-Observe each received 6 daily trials of T→L and N. Trials occurred with a mean interval of  $5 \pm 3$  min during each daily 60-min session.

*Phase 2: First-order conditioning.* Days 6-7: All rats received 12 trials of L→F in each daily 60-min session with a mean interval of  $5 \pm 3$  min.

*Testing.* Levers were extended into the chambers for testing and the bulb on which L had been presented during training was removed from the experimental chamber. (A pilot experiment found it necessary to remove the light bulb on which L was

presented during training to produce reliable responding to T after causal-chain training (S4). Thus, the bulb was removed from the apparatus prior to testing for all subjects in Experiments 2a and 2b to equate conditions among all groups).

Days 8-9: In each 30-min session, rats in Groups Common-Cause-Intervene and Chain-Intervene received presentations of T each time the lever was pressed. Rats in Groups Common-Cause-Observe and Chain-Observe received presentations of T yoked to the number of presentations of T by rats in Groups Common-Cause-Intervene and Chain-Intervene, respectively, in the same manner as described in Experiment 1. We recorded the number of nose pokes during T, during the 10s after T (Post-T Interval 1), and during the 10s after Post-T Interval 1 (Post-T Interval 2). We analyzed nose pokes during T and during Post-T Interval 2. Due to a programming error, lever press data were lost for all subjects in Experiments 2a and 2b preventing us from analyzing lever press data in these experiments. One subject from Group Chain-Observe was eliminated for meeting the elimination criterion on acquisition of responding to L during acquisition.

### *Results*

No group differences in mean number of nose pokes during the background were found, thus, baseline responding did not contribute to responding to the test stimulus (Means  $\pm$  SEM = 11895  $\pm$  1664, 11081  $\pm$  1206, 13096  $\pm$  1256, and 11642  $\pm$  2756 for Conditions Common-Cause-Intervene, Common-Cause-Observe, Chain-Intervene, and Chain-Observe, respectively). An ANOVA on Training Model (Common-Cause vs. Chain) and Testing Condition (Intervene vs. Observe) found neither main effects nor an interaction,  $F_s(1, 35) < 1.0$ .

A two-way ANOVA conducted on mean nose pokes during T revealed a main effect of Testing,  $F(1, 35) = 12.65, P < 0.01$ , but no interaction between Training Model and Testing. Although subjects nose poked numerically less in Group Chain-Observe than in Group Common-Cause-Observe, this difference was not significant,  $F(1, 35) = 1.54, P > .22$ . However, responding in Group Chain-Intervene also did not differ from Group Common-Cause-Intervene,  $F < 1.0$ .

A similar ANOVA conducted on mean nose pokes during Post-T Interval 2 revealed a main effect of Testing,  $F(1, 35) = 6.43, P < 0.05$ , and a Training Model X Test interaction,  $F(1, 35) = 4.86, P < 0.05$ . Planned comparisons revealed that rats in Group Common-Cause-Intervene nose poked less during Post-T Interval 2 than did rats in Group Common-Cause-Observe,  $F(1, 35) = 11.55, P < 0.01$ . No difference was found between Groups Chain-Intervene and Chain-Observe,  $F < 1.0$ . Subjects in Group Chain-Observe nose poked less than those in Group Common-Cause-Observe,  $F(1, 35) = 4.42, P < .05$ . No difference in number of nose pokes was found between Groups Chain-Intervene and Common-Cause-Intervene,  $F = 1.0$ . It is not clear why the Chain groups should make fewer nose pokes than Group Common-Cause-Observe. Nevertheless, it is important to document learning of the chain. Thus, Experiment 2b compared responding in the Chain groups to that of an unpaired cue.

#### Experiment 2b

*Subjects, Apparatus, and Magazine training.* As in Experiment 2a.

*Phase 1: Sensory preconditioning.* As in Experiment 2a for Groups Chain-Intervene and Chain-Observe. Rats in Groups Unpaired-Intervene and Unpaired-Observe

received 6 trials each of T, L, and N separately with a mean interval of  $5 \pm 3$  min during each daily 60-min session.

*Phase 2: First-order conditioning.* As in Experiment 2a.

*Testing.* Days 8-11: T was presented in the following manner in each 30-min session. Groups Chain-Intervene and Chain-Observe received treatment identical to Experiment 2a. Rats in Groups Unpaired-Intervene and Unpaired-Observe received presentations of T when rats in Group Unpaired-Intervene lever pressed. Data recorded as in Experiment 2a. One subject from Group Unpaired-Observe was removed for meeting the elimination criterion. Data from one rat from Group Chain-Intervene and two from Group Unpaired-Intervene were lost due to equipment failure.

### *Results*

Mean number of nose pokes during the background did not differ among groups—indicating no contribution of baseline levels of responding to the test stimulus (Means =  $920 \pm 233$ ,  $1211 \pm 224$ ,  $1173 \pm 258$ , and  $1598 \pm 286$  for Conditions Chain-Intervene, Chain-Observe, Unpaired-Intervene, and Unpaired-Observe, respectively). A two-way ANOVA on Training Model (Chain vs. Unpaired) and Testing Condition (Intervene vs. Observe) found neither main effects nor an interaction,  $F_s(1, 33) < 1.53$ .

A two-way ANOVA conducted on nose poking during T revealed a marginal effect of Training Model,  $F(1, 33) = 2.96$ ,  $P = 0.09$ , but neither a main effect of Testing nor an interaction between Training Model and Testing. A similar ANOVA conducted on mean nose pokes during the Post-T Interval 2 revealed a main effect of Training Model,

$F(1, 33) = 8.32, P < 0.01$ , but neither a main effect of Testing nor a Training Model X Testing interaction.

The main effect of training model during Post-T Interval 2 demonstrates significant nose poking in the Chain groups relative to the Unpaired groups, establishing the effectiveness of causal-chain training to establish a causal chain representation. The findings also demonstrate that rats exhibit a sensitivity to the T→L and L→F temporal relationships underlying the causal-chain (S5). Thus, rats in the Chain groups expected F during Post-T Interval 2, but not before.

### **Supporting References**

S1. B. F. Skinner, *The behavior of organisms: an experimental analysis*, (Appleton-Century Company, Inc., New York, 1938).

S2. N. Winterbauer, B. Balleine, *unpublished*.

S3. W.L. Inglis, M. C. Olmstead, T. W. Robbins, *Behav. Neuro.* **114**, 285 (2000)

S4. We thank K. Holyoak for suggesting this procedure.

S5. H. I. Savastano, R. R. Miller, *Behavioural Processes* **44**, 147 (1998).

S6. All procedures involved in this research met the approval of UCLA's animal care and followed institutional guidelines.