

Supporting Information for

**A simple method that doubles learning speed for mice in
touchscreen-based visual discrimination**

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Supplemental Figure Legends 1 to 8

Supplemental Table 1

Titles of Supplemental Movies 1 to 2

(Supplemental_figures_kiyama_et_al.pdf, Movie S1.mp4, Movie S2.mp4)

Supplemental Figure Legends

Figure S1. (A) Experimental schedule for the 1-pair VD task. The initial learning phase is conducted following pre-training, followed by a reversal learning phase. (B) Stimulus patterns used in the 1-pair VD task, with vertical stripes as S+ and horizontal stripes as S- during initial learning. In the reversal learning phase, the S+ and S- were switched. (C) Performance of the 1-pair VD task during initial and reversal learning with the original one-touch protocol (1T). Small symbols (bordered for males and filled for females) and lines represent individual animals' performance, while large blue circles represent the mean across animals. N = 7 mice. (D) Task flowchart for the three-touch (3T) protocol. Mice were required to touch the same stimulus three times in succession to register a choice.

Figure S2. Task performance of males and females in the 1-pair VD task during initial learning with the m3T protocol. Small symbols (bordered for males and filled for females) and lines represent individual animals' performance. Large open and filled red circles indicate the mean across all males and all females, respectively. Repeated-measures two-way ANOVA revealed no significant main effect of sex ($p = 0.751$) and no significant interaction between sex and training sessions ($p = 0.7136$).

Figure S3. Nose poking after the final decision with 3T and m3T. (A) Latency to nose poke after correct judgements. (B) Latency to nose poke after false judgements. (C) The percentage of trials in which the mouse did not nose poke during the 10-second ITI following a false judgement. ***indicates $p < 0.001$.

Figure S4. Evaluation of side bias in mice during the 1-pair VD task. (A) Absolute side bias index (ASBI) during initial and reversal learning with the 1T and m3T protocols.

ASBI is defined as:

$$ASBI = \frac{|L-R|}{(L+R)}$$

where L and R represent the number of left and right choices during a session, respectively.

An ASBI value was calculated for each animal for each session. A session with a completely spatially biased response results in an ASBI of 1, whereas a perfectly balanced session scores 0. (B) Side bias index (SBI) during initial and reversal learning with the

1T and m3T protocols. SBI is defined as:

$$SBI = \frac{(L-R)}{(L+R)}$$

The mean SBI across animals was used to assess whether mice had a general preference for either side.

Figure S5. Transverse patterning task with the m3T protocol. (A) Experimental schedule.

The transverse patterning task was conducted following the pre-training phase and the initial learning of the 1-pair VD task. This task consisted of two phases, with Phase 2 beginning after the completion of 17 sessions in Phase 1. (B) Stimulus patterns and their relationships in the transverse patterning task. In Phase 1, geometric patterns were used, whereas in Phase 2, two natural scene images were employed. (C) Task flowchart for the modified three-touch (m3T) protocol. (D) Performance of the transverse patterning task with the m3T protocol. Small bordered symbols represent individual males, while small filled symbols indicate individual females. Large green circles indicate the mean across animals (N = 4).

Figure S6. Further modification of the m3T protocol to eliminate auditory cues for intermediate touches by using a neutral sound (the m3Tn protocol). (A) Task flowchart for the m3Tn protocol. Beep A is a sound distinct from the tone used for correct responses and the buzz used for incorrect responses. (B) Experimental schedule for the initial learning of the 1-pair VD task with the m3Tn protocol. (C) Performance of the 1-pair VD task during initial learning with the m3Tn protocol. Small bordered symbols represent individual males, while small filled symbols indicate individual females. Large purple circles indicate the mean across animals. (D) Number of sessions required to reach criterion with the m3Tn protocol. (E) Highest performance of individual mice with the m3Tn protocol.

Figure S7. (A) Sessions to the criterion for initial (left) and reversal (right) learning in the 3-pair VD task with different protocols. NR denotes “not reaching the criterion” by Session 20. Horizontal bars indicate median values. $**p < 0.01$; $*p < 0.05$, determined by Dunn’s multiple comparisons test. (B) Highest performance of individual subjects in initial (left) and reversal (right) learning. $**p < 0.01$; $*p < 0.05$, determined by Tukey’s multiple comparisons test. (C) Performance in the first session after each reversal of the 3-pair serial reversal VD task with m3T. (D) Sessions to the criterion during repeated reversals, showing the across-animal average. Error bars indicate s.e.m.

Figure S8. Internal state estimation across multiple-touch protocols. (A) Simulated learning curves generated from the Q -learning model to match observed behavioral data (Fig. 4B). The model parameters were estimated from the simulation learning curves: decay constant α (B), learning rate β (C), and perseverative traits τ (D).

Supplemental Table 1. Number and sex of animals in individual graphs

Figure	Group(s)	Sex	n
Figure 1C, D, E	1T	Male	9
		Female	0
	2T	Male	9
		Female	0
	3T	Male	7
		Female	0
Figure 2B, C, D	1T	Male	4
		Female	3 ^a
	m3T	Male	3
		Female	2
Figure 3C	Same cohort as the m3T group in Figure 2		
Figure 4B, C	1T	Male	5
		Female	0
	2T	Male	9 ^b
		Female	0
	3T	Male	7 ^b
		Female	0
	m3T	Male	8
		Female	0
Figure S1C	Same cohort as the 1T group in Figure 2		
Figure S2	m3T	Male	9 ^c
		Female	8 ^c
Figure S3	Same cohorts as the 3T and m3T groups in Figure 4		
Figure S4	Same cohorts as the 1T group in Figure 2 and the m3T group in Figure S2		
Figure S5	m3T	Male	2
		Female	2
Figure S6	m3Tn	Male	3
		Female	3
Figure S7	Same cohorts as in Figure 4		

a, One animal failed to reach the criterion and was omitted from the reversal learning analysis.

b, Same cohorts as shown in Figure 1.

c, Cohort includes the m3T group shown in Figure 2 and Figure S5.

Titles of Supplemental Movies

Movie S1. An example of a mouse performing the 1-pair VD task in the 1T protocol.

Movie S2. An example of a mouse performing the 1-pair VD task in the m3T protocol.

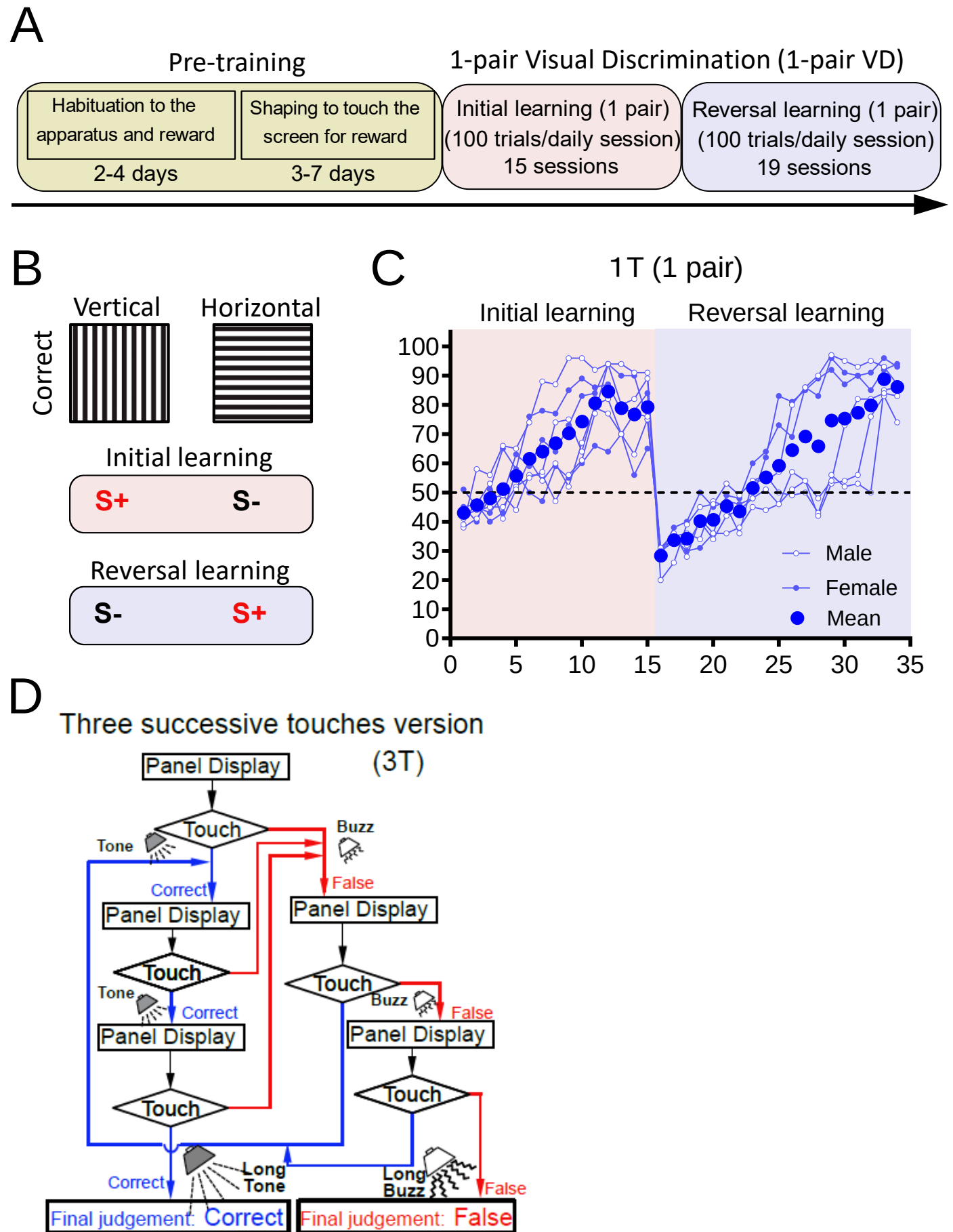
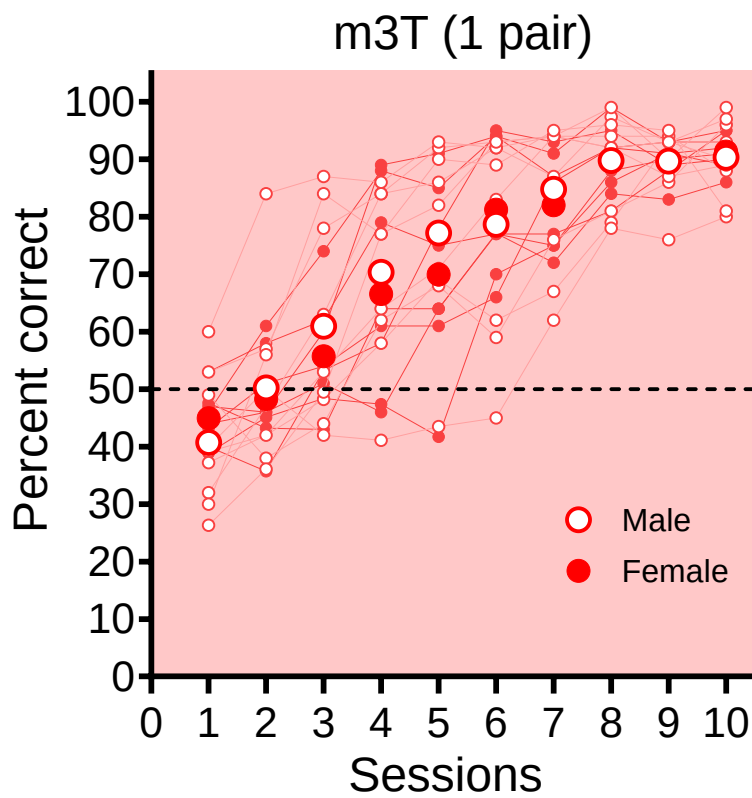


Figure S1



Repeated Measures two-way ANOVA

Main effect of sex: $F_{(1, 15)} = 0.1044$, $p = 0.751$

Main effect of session: $F_{(9, 135)} = 66.91$, $p < 0.0001$

Interaction between Sex and Session: $F_{(9, 135)} = 0.6938$, $p = 0.7136$

Figure S2

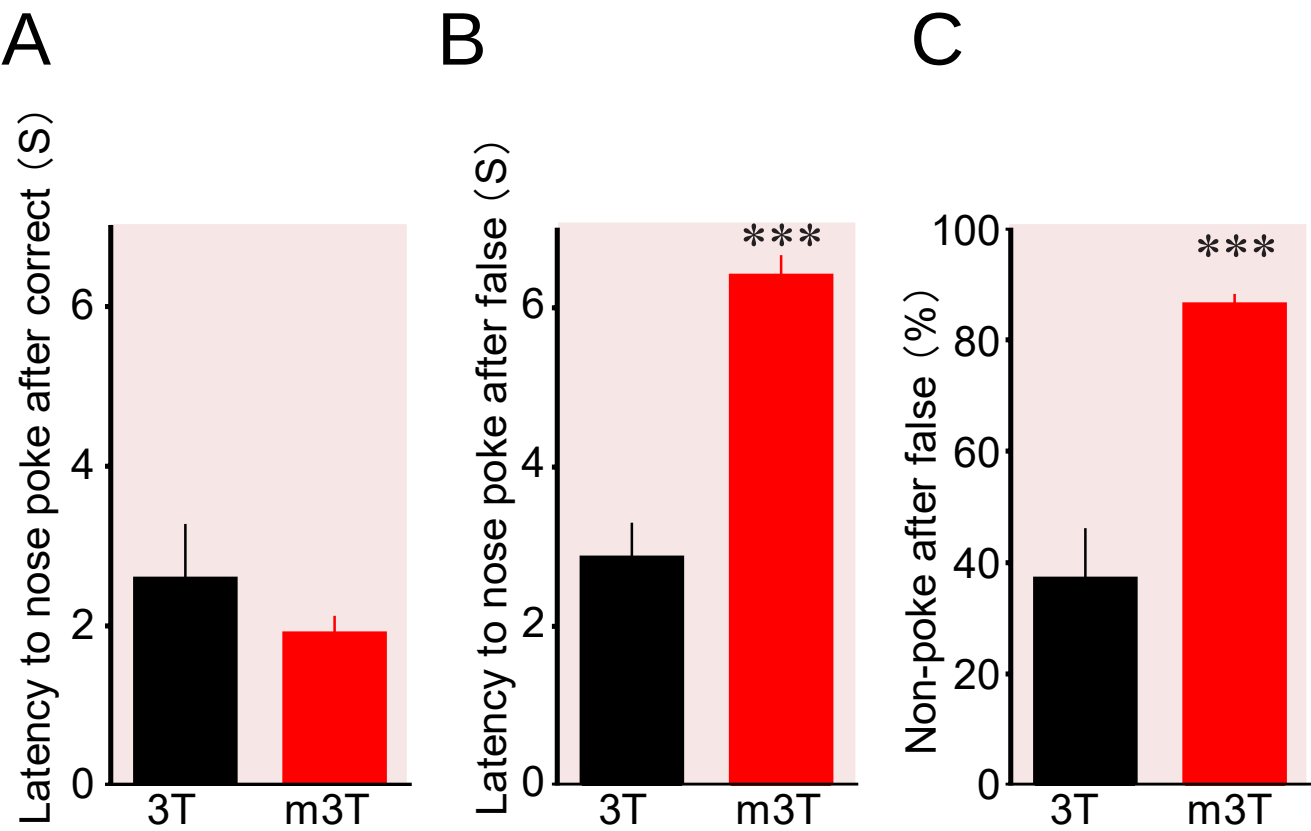
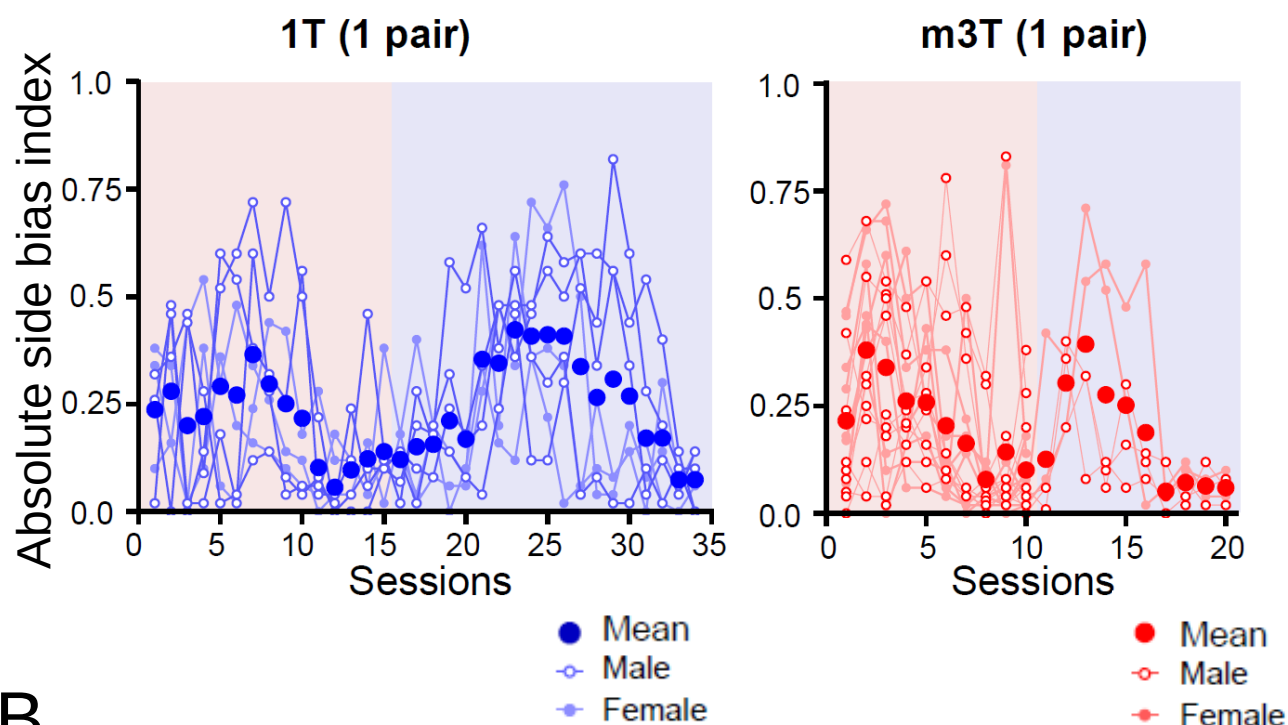


Figure S3

A



B

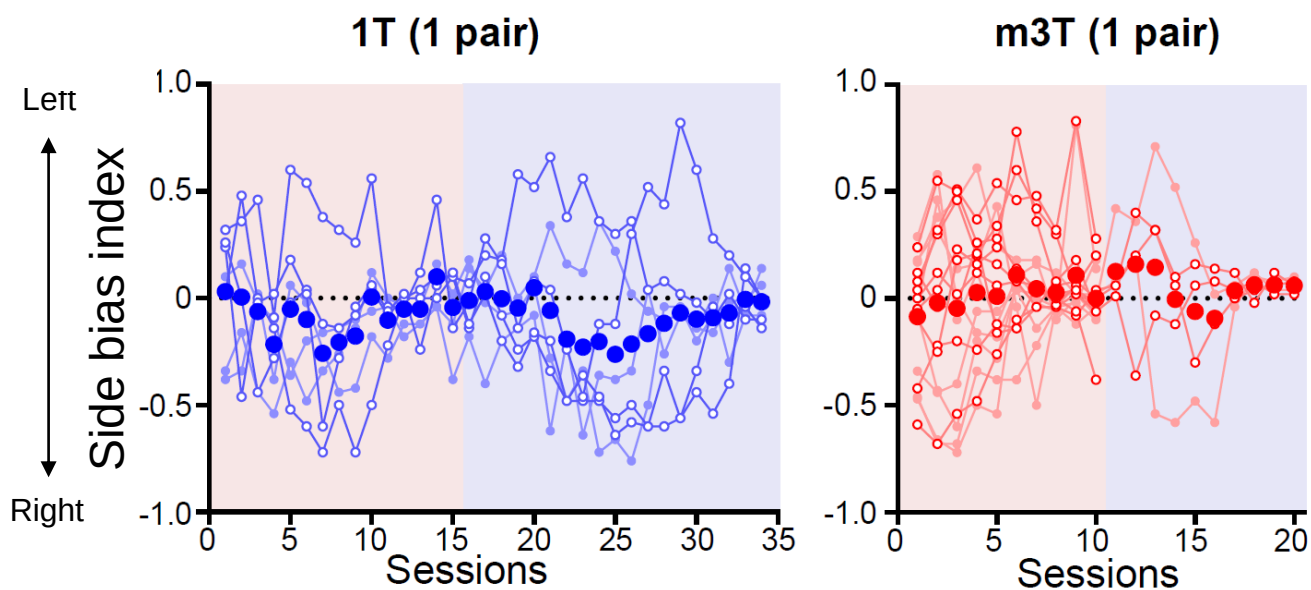
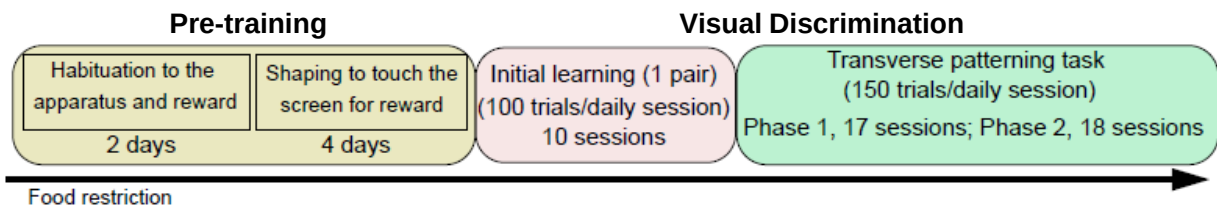


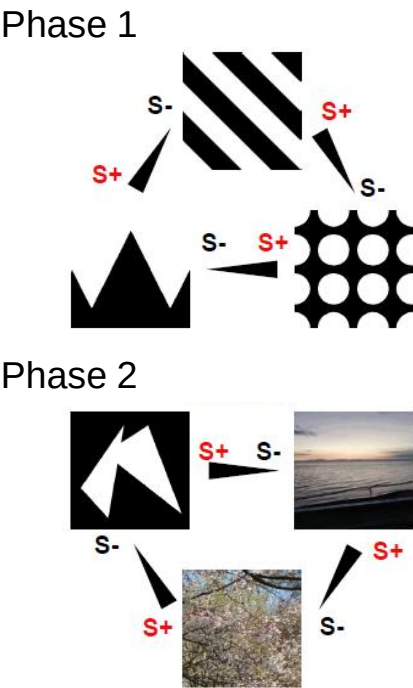
Figure S4

A

with the m3T protocol

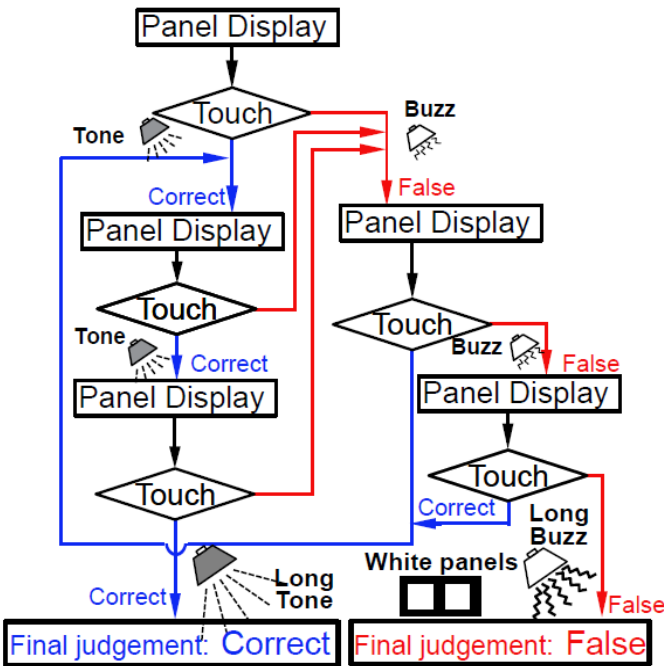


B



C

Modified three successive touches version (m3T)



D

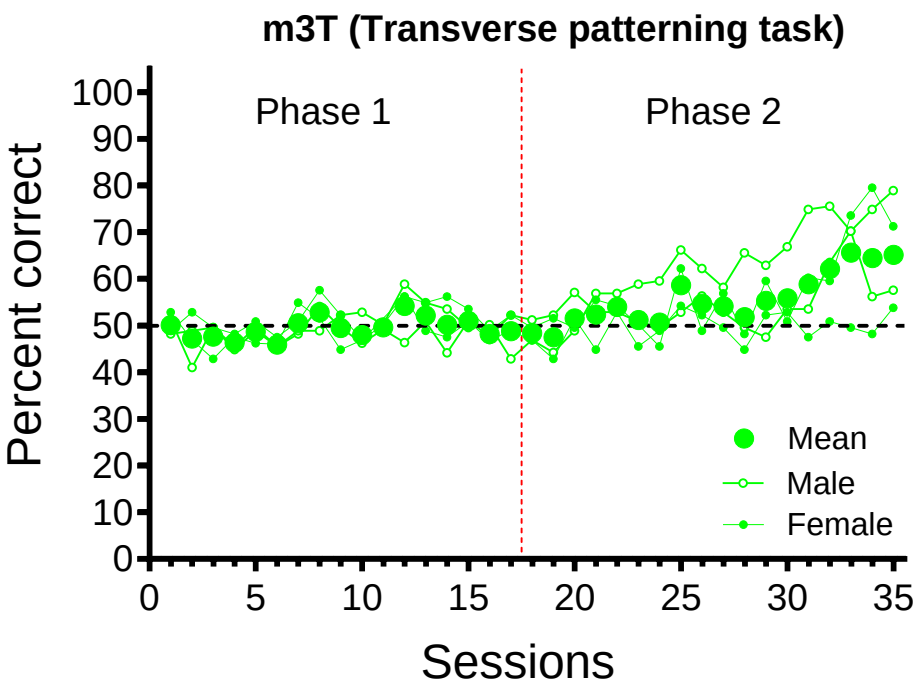


Figure S5

A Three successive touches with **neutral tone** (m3Tn)

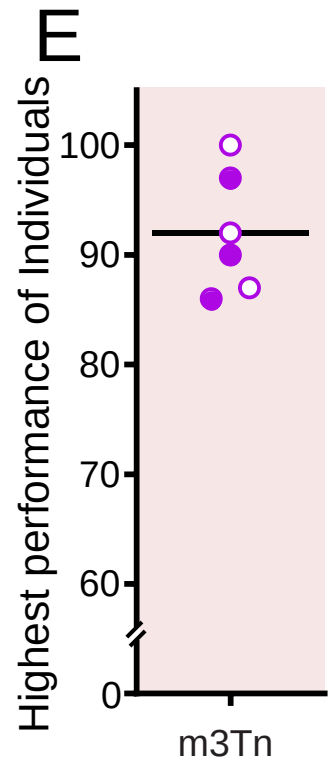
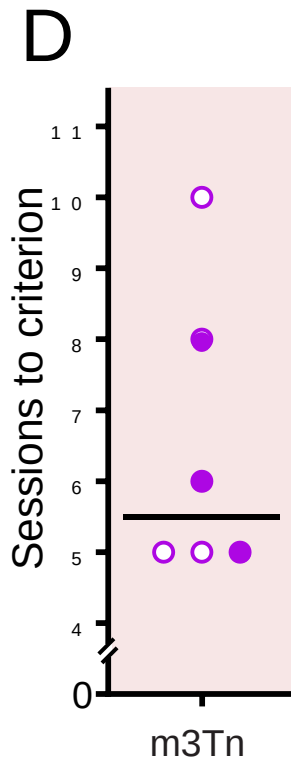
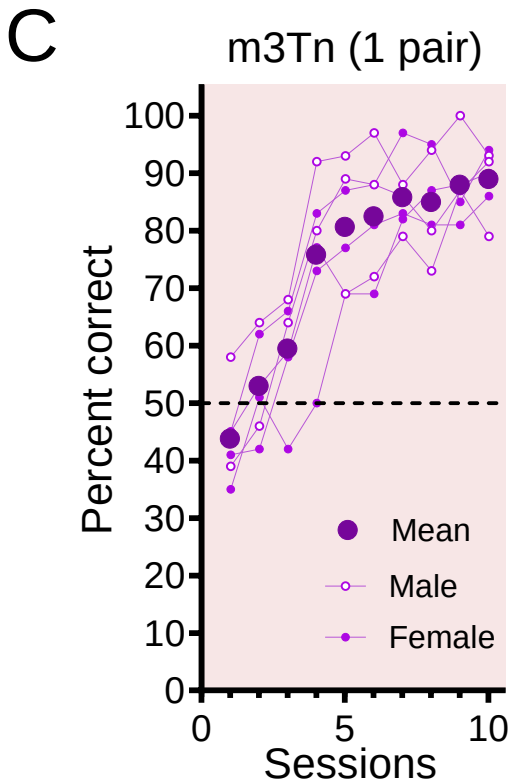
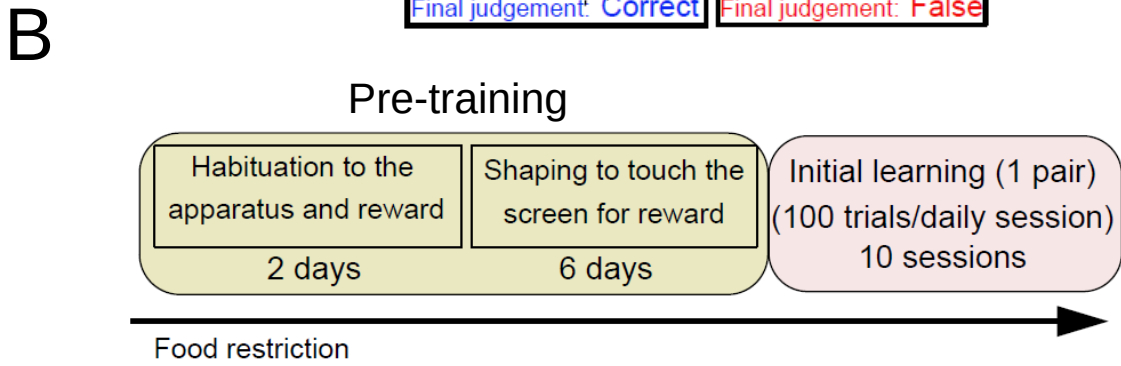
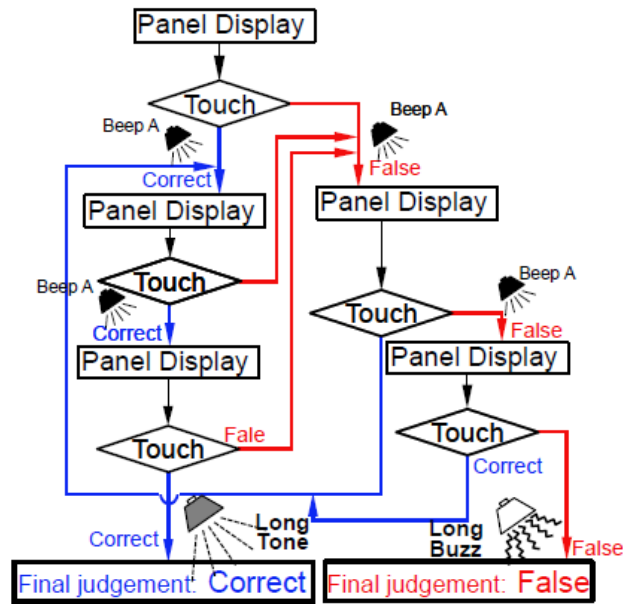
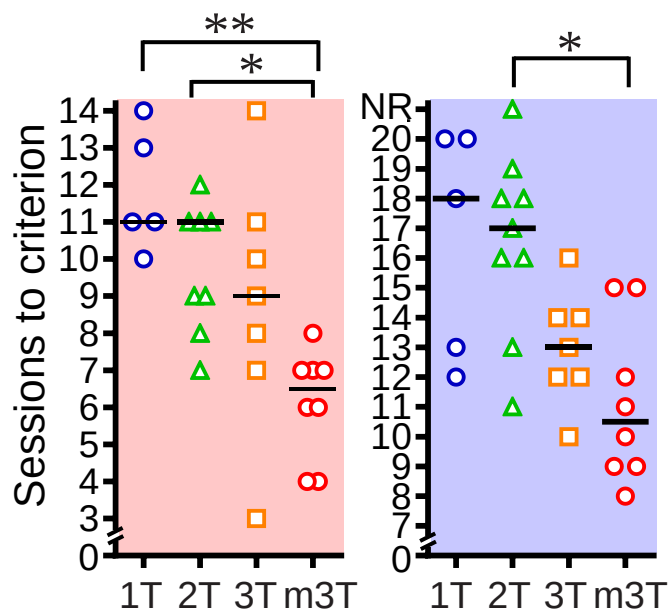
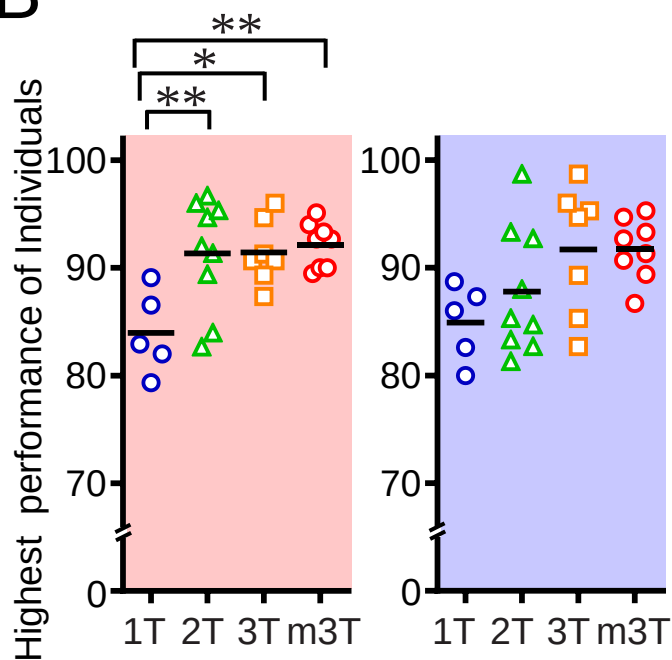


Figure S6

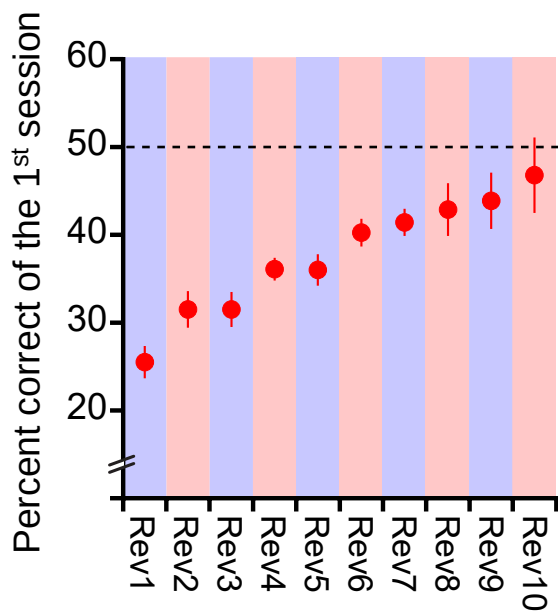
A



B



C



D

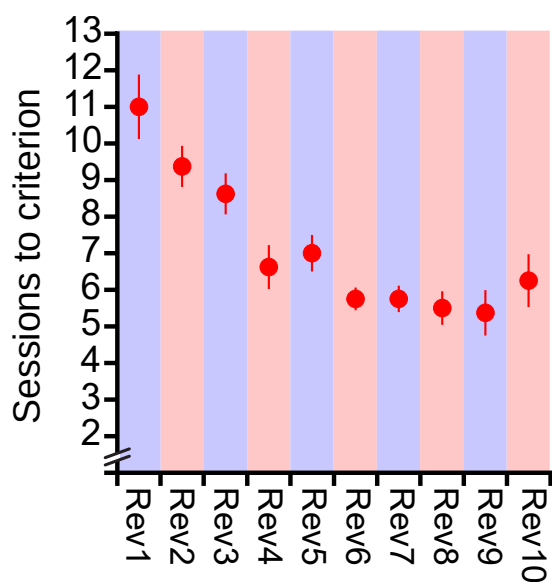
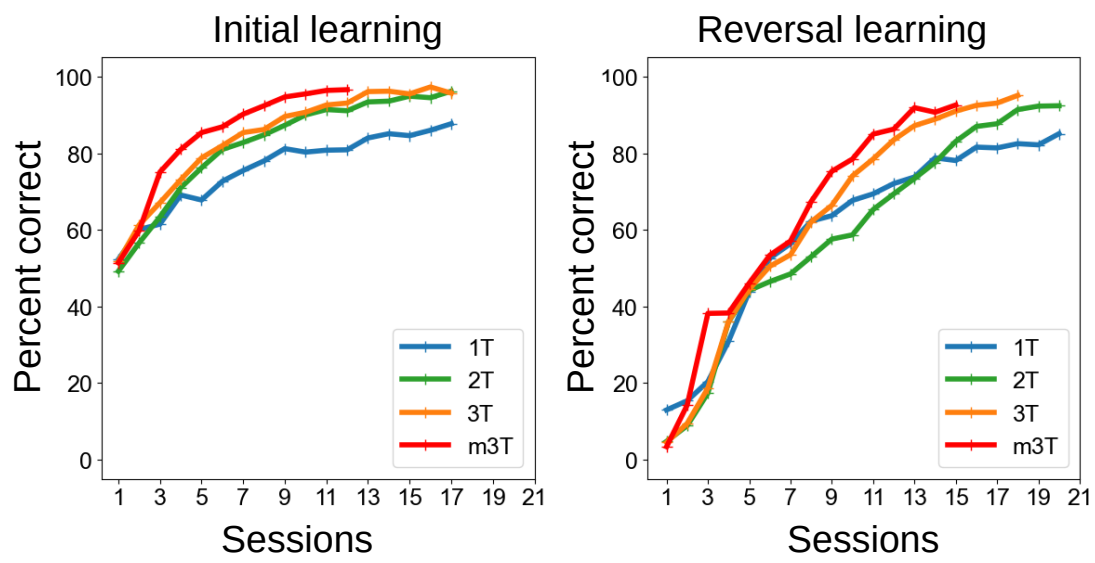
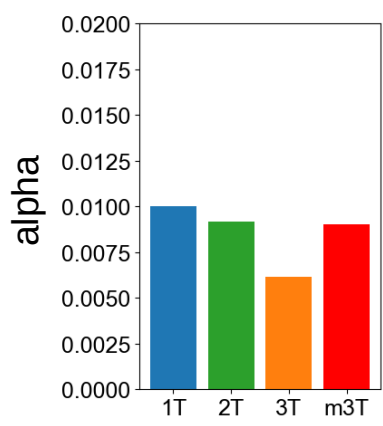


Figure S7

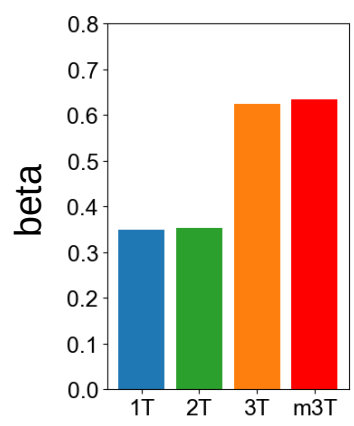
A



B



C



D

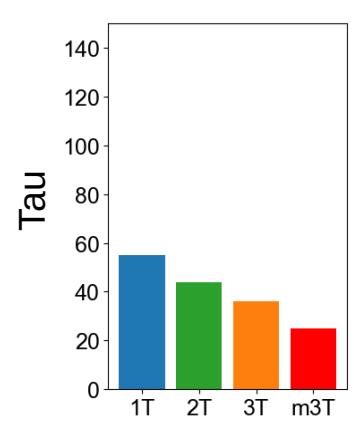


Figure S8