

1 Research Paper

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4 **A self-initiated cue-reward learning procedure for neural**  
5 **recording in rodents**

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38 **Abstract**

39 Background: Single-unit recording in Pavlovian conditioning tasks requires the use of  
40 within-subject designs as well as sampling a considerable number of trials per trial type  
41 and session, which increases the total trial count. Pavlovian conditioning, on the other  
42 hand, requires a long average intertrial interval (ITI) relative to cue duration for cue-  
43 specific learning to occur. These requirements combined can make the session duration  
44 unfeasibly long.

45 New Method: To circumvent this issue, we developed a self-initiated variant of the  
46 Pavlovian magazine-approach procedure in rodents. Unlike the standard procedure,  
47 where the animals passively receive the trials, the self-initiated procedure grants  
48 animals agency to self-administer and self-pace trials from a predetermined,  
49 pseudorandomized list. Critically, whereas in the standard procedure the typical ITI is in  
50 the order of minutes, our procedure uses a much shorter ITI (10 s).

51 Results: Despite such a short ITI, discrimination learning in the self-initiated procedure  
52 is comparable to that observed in the standard procedure with a typical ITI, and superior  
53 to that observed in the standard procedure with an equally short ITI.

54 Comparison with Existing Method(s): The self-initiated procedure permits delivering 100  
55 trials in a ~1-h session, almost doubling the number of trials safely attainable over that  
56 period with the standard procedure.

57 Conclusions: The self-initiated procedure enhances the collection of neural correlates of  
58 cue-reward learning while producing good discrimination performance. Other  
59 advantages for neural recording studies include ensuring that at the start of each trial

60 the animal is engaged, attentive and in the same location within the conditioning  
61 chamber.

## 62 **1. Introduction**

63 Progress in behavioral neuroscience rests on the foundation of well-controlled  
64 behavioral designs capable of isolating the cognitive process of interest and yielding  
65 replicable results (Krakauer et al., 2017). However, adapting traditional behavioral  
66 procedures to meet the requirements of neuroscience techniques may demand some  
67 ingenuity. One class of challenge stems from the fact that experimental parameters  
68 favorable to the cognitive process under investigation may conflict with those that best  
69 suit the neuroscience technique at hand. This conflict becomes apparent, for instance,  
70 when investigating the neural correlates of cue-reward learning with neural recording  
71 techniques such as in-vivo electrophysiological recording and calcium imaging. Here,  
72 we introduce a self-initiated, self-paced conditioning procedure for rodents specifically  
73 designed to enhance the acquisition of neural data in such scenarios.

74 The mechanisms of cue-reward learning have been dissected by learning  
75 theorists using Pavlovian conditioning procedures (Mackintosh, 1974; Kehoe & Macrae,  
76 2002), which have led and continue to lead to highly influential findings in neuroscience  
77 (e.g., Hawkins et al. 1983; Kim et al., 1998; Maren, 2001; Schultz & Dickinson, 2000;  
78 Waelti et al., 2001; Holland, 1997). In the rat, one such procedure is the conditioned  
79 magazine approach (e.g., Boakes, 1977; Harris et al., 2013), in which animals receive  
80 presentations of certain cues or conditioned stimuli (CSs; typically, visual or auditory)  
81 followed when appropriate by the delivery of a reward or an unconditioned stimulus (US;  
82 e.g., sucrose solution). Reward expectancies are typically quantified by measuring the

83 total number of head-entries or the cumulative percentage of time spent in the reward  
84 magazine during the CS before the US is delivered (Gottlieb, 2005). A discrimination is  
85 said to emerge as the rat responds more in the presence of rewarded than unrewarded  
86 cues. Critically, as in other Pavlovian procedures (Prokasy & Ebel, 1964; Salafia et al.,  
87 1973; Terrace et al., 1975; Domjan, 1980; Gibbon & Balsam, 1981; Yin et al., 1994;  
88 Barella, 1999), better performance is observed with spaced rather than massed trial  
89 presentations; that is, when the intertrial interval (ITI) is sufficiently long relative to the  
90 duration of the cues (i.e., the trial-spacing effect; Lattal, 1999; Holland, 2000).

91         Although scheduling a long ITI benefits learning, it can be problematic in neural  
92 recording studies. To illustrate why, consider the results of a series of bootstrap  
93 analyses conducted on in-vivo electrophysiological data recorded in the rat orbitofrontal  
94 cortex (Fig. 1 and Supplemental Materials). The data were collected during the CS  
95 epochs of a well-trained visual discrimination using the novel reward-learning procedure  
96 introduced here. The top panels show that the number of neurons that significantly  
97 discriminate between rewarded and unrewarded trials steadily increases as more trials  
98 are sampled. The bottom panels bolster this point by showing that the statistical power  
99 observed for each neuron in the same analysis is also a monotonically-increasing  
100 function of the number of trials sampled. The story told by this figure will be familiar to  
101 many in-vivo electrophysiologists: single-unit recording requires the presentation of a  
102 sizeable number of trials per trial type in order to average out the trial-to-trial variability  
103 inherent to neural data.

104         This requirement, when combined with that of a long ITI in Pavlovian procedures,  
105 will produce lengthy neural recording sessions—often unfeasibly so once the

106 experimenter ventures beyond basic discrimination designs. To compound the issue,  
107 neural recording studies demand the use of within-subject designs in order to compare  
108 neural responses between experimental and control cues, further contributing to  
109 elevating the total trial count in a session. This makes examining the neural bases of  
110 discrimination, categorization and rule learning difficult for the in-vivo electrophysiologist  
111 working with rodents. Such scenarios involve more complex experimental designs,  
112 leaving the experimenter with a hard choice between shortening the ITI, which can  
113 jeopardize learning, and reducing the number of trials at the peril of insufficiently  
114 sampling neural activity.

115         To circumvent this choice, we developed a variant of the Pavlovian conditioned  
116 magazine-approach procedure we have dubbed the *self-initiated conditioned magazine*  
117 *approach* (SICMA) procedure. Unlike the standard procedure, where the rat passively  
118 receives the trials, in SICMA it falls upon the animal to initiate each trial by performing a  
119 separate response upon receiving a cue signaling trial availability. Because the ITI is  
120 only 10 s on average, SICMA permits packing 100 trials in a ~1 h session, almost  
121 doubling the number of trials safely attainable in that time with the standard Pavlovian  
122 procedure. Crucially, despite such a short ITI, our results show that performance in  
123 SICMA is comparable to that observed in the standard procedure (Experiment 1), and  
124 superior to that observed in a yoked Pavlovian group (Experiment 2). In addition, we  
125 provide evidence that magazine-approach responses to cues trained with the SICMA  
126 procedure readily transfer when the cues are presented in a standard Pavlovian fashion  
127 (Experiment 3). Thus, SICMA affords the in-vivo electrophysiologist an opportunity to

128 efficiently examine the neural underpinnings of cue-reward learning using complex  
129 discrimination designs.

130

## 131 **2. Experiment 1: comparison of SICMA with the standard Pavlovian magazine-** 132 **approach procedure**

133

134 The goal of this experiment was to compare a group of rats trained with the SICMA  
135 procedure (labeled SICMA) with another one trained with the standard Pavlovian  
136 magazine-approach method (labeled Pav) in their ability to solve two discriminations  
137 involving visual and auditory cues. The experimental parameters used in the Pav group  
138 (e.g., ITI and CS durations) were known from prior unpublished work from our  
139 laboratory to produce good discrimination performance.

### 140 2.1. Materials and Methods

#### 141 *2.1.1. Animals*

142 All animal care and experimental procedures were conducted according to the National  
143 Institutes of Health's *Guide for the Care and the Use of Laboratory Animals*, and  
144 approved by the Brooklyn College Institutional Animal Care and Use Committee  
145 (Protocol #303). Subjects were 32 experimentally-naïve, adult Long-Evans rats (16  
146 males and 16 females) bred at Brooklyn College from commercially available  
147 populations (Charles River laboratories). At the start of the experiment, all rats were  
148 approximately 90 (+/- 7) days old and their weights ranged between 244 and 271 g for  
149 females and between 317 and 340 g for males. They were housed individually in  
150 standard clear-plastic tubs (10.5 in. × 19 in. × 8 in) with woodchip bedding in a colony

151 room on a 14:10 light/dark schedule. Behavioral sessions were conducted between 3-6  
152 hours after the onset of the light phase of the cycle. Throughout training, food was  
153 provided *ad libitum* but water access was restricted to 1 h/day immediately after each  
154 experimental session.

### 155 2.1.2. Apparatus

156 Behavioral training was conducted in eight standard conditioning chambers (Med  
157 Associates Inc., St. Albans, VT, USA) measuring 32 cm in length, 25 cm in width and 33  
158 cm in height, and comprising a stainless-steel grid floor, a Perspex front door, back wall,  
159 and ceiling, and modular left and right walls. Each chamber was enclosed in a ventilated  
160 sound-attenuating cubicle (74 cm x 45 cm x 60 cm) that provided a background noise  
161 level of ~50 dB. A schematic depiction of the interior of the chambers is shown in Figure  
162 S1 (Supplemental Materials, Section S2.1). All reported locations of stimulus and  
163 response apparatus were measured from the grid floor of the conditioning chamber to  
164 the lowest point or edge of the apparatus. The left wall of the chamber housed two white  
165 jewel lamps 2.5 cm in diameter (28V DC, 100 mA) located 9.3 cm above the grid floor  
166 on the left and right panels, as well as a speaker (7 cm x 8.2 cm) located 20.6 cm above  
167 the grid floor on the right panel and connected to a dedicated tone generator capable of  
168 delivering a 12-kHz, 70-dB tone. The right wall housed a third white jewel lamp (28V  
169 DC, 100 mA) 2.5 cm in diameter, located 17.2 cm above the grid floor on the center  
170 panel, as well as a speaker located 24.8 cm above the grid floor on the left panel and  
171 connected to a dedicated tone generator capable of delivering a 70-dB white noise. The  
172 right wall also housed a circular noseport 2.6 cm in diameter located on the center panel  
173 4.6 cm above the grid floor, equipped with a yellow LED light and an infrared sensor for

174 detecting nose entries. This noseport was flanked by a recessed liquid reward  
175 magazine (aperture: 5.1 cm x 15.2 cm) located on the right panel 1.6 cm above the grid  
176 floor. This magazine was equipped with an infrared sensor for detecting head entries,  
177 and connected to a liquid dipper that could deliver a 0.04 cc droplet of a 10% sucrose  
178 solution. The chambers remained dark throughout the experimental session except  
179 during presentations of the visual stimuli. In the same room was a computer running  
180 Med PC IV software (Med Associates Inc., St. Albans, VT, USA) on Windows OS which  
181 controlled and automatically recorded all experimental events via a Fader Control  
182 Interface.

183

### 184 *2.1.3. Procedure*

#### 185 *2.1.3.1. Magazine training and shaping*

186 Animals were first randomly assigned to the SICMA and Pav groups (16 rats in each,  
187 gender balanced). Each session began with a 2-min acclimation period in the  
188 conditioning chambers. Rats were initially magazine-trained in a 1-h session to retrieve  
189 up to 60 deliveries of a 10% sucrose reward at the dipper magazine. For the first 10  
190 trials, the reward was made available for 30 s every 30 s; for the second 20 trials, it was  
191 available for 20 s every 40 s; and finally, for the last 30 trials, it was available for 10 s  
192 every 50 s.

193 Rats in the SICMA group then went on to receive five additional shaping  
194 sessions. On the first of these sessions, the noseport light was turned on for a  
195 maximum of 20 s, during which a nose poke immediately resulted in the termination of  
196 the noseport light and the onset of the sucrose reward, which remained available for 10



197 s. Trials were separated by a 10 s variable ITI (range: 5-15 s). Over the following four  
198 shaping sessions, we introduced and progressively increased a delay (2, 4, 6, and 8 s)  
199 between the rat's response at the port and reward delivery, during which the noseport  
200 light would flash at a 1-Hz frequency (on for 0.5 s, off for 0.5 s). Concurrently, reward  
201 availability was progressively shortened (8, 6, 4, and 3 s).

202

### 203 *2.1.3.2. Trial structure*

204 Fig. 2 depicts the basic trial structure in the SICMA procedure. As during shaping, rats  
205 in the SICMA group were still required to self-initiate trials in this phase by responding at  
206 the lit-up noseport during the 20-s periods of trial availability. Failure to respond resulted  
207 in the noseport light coming off and the trial being repeated after a short ITI averaging  
208 10 s and ranging 5-15 s. In contrast, performing a nose-poke response immediately  
209 terminated the noseport light and triggered the onset of one of four possible 10-s CSs.  
210 Reinforced trials culminated in 3 s of access to the sucrose reward, followed by a short  
211 ITI (average 10 s; range: 5-15 s). In contrast, rats in the Pav group received the 10-s  
212 CSs in the standard Pavlovian conditioning manner (i.e., noncontingent on any  
213 response), followed, whenever reinforced, by the same reward used in the SICMA  
214 group. The ITI in the Pav group was 60 s on average (range: 40-80 s).

215

### 216 *2.1.3.3. Discrimination training*

217 Although any discrimination can be imbedded in SICMA, the experiments reported here  
218 involved two discriminations, one involving two visual CSs (V1 & V2, counterbalanced)  
219 and the other two auditory ones (A1 & A2, counterbalanced). A table containing the

220 details of the stimulus counterbalancing can be found Section S3 of the Supplemental  
221 Materials (Table S1). One visual CS was constructed by flashing the two jewel lamps on  
222 the left wall alternately at a 2-Hz frequency (on for 0.25 s, off for 0.25 s). The second  
223 visual CS was provided by the steady illumination of the white jewel lamp located on the  
224 right wall. The two auditory CSs were provided by a 12-kHz, 70-dB tone played from the  
225 speaker on the left wall and a 70-dB white noise played from the speaker on the right  
226 wall. The probability of reinforcement varied across the CSs, with V1 and V2 reinforced  
227 on 100% and 0% of trials, respectively, and A1 and A2 reinforced pseudorandomly on  
228 75% and 25% of trials, respectively. In the SICMA group, each session ended when the  
229 rat completed 96 trials or else it timed out at 90 min. Rats in the Pav group received a  
230 total of 64 trials per session. Although this may seem an unfair comparison from the  
231 viewpoint of trial-centered theories of predictive learning (e.g., Rescorla & Wagner,  
232 1972; Wagner, 1981; Stout & Miller, 2007), evidence indicates that the number of trials  
233 in a session has no measurable effect on the rate of acquisition when assessed—as in  
234 the present case—in between-subject designs (Gottlieb, 2008).

235

#### 236 *2.1.3.4. Statistical analysis*

237 For this and the remaining experiments, we used the percentage of time each rat spent  
238 in the reward magazine during the cues, a widely used measure of conditioned  
239 responding (e.g., Kaye & Pearce, 1984; Hunt & Campbell, 1997; Holland, 1999;  
240 Gottlieb, 2005). We chose this dependent variable above the other conventional  
241 measure—the rate of head entries per minute—because we have observed that in  
242 SICMA-trained rats it provides a more sensitive index of discrimination learning. This

243 can be readily appreciated in Figure S2 (Section S2.2 of Supplemental Materials), which  
244 depicts the count of rats in the SICMA and Pav groups across the last two sessions of  
245 Experiment 1 as a function of the mean number of head entries during cues V1 (left  
246 panel) and A1 (right panel)—the cues with the highest reinforcement probability within  
247 either sensory modality.

248 The figure shows rather different response distributions for each cue between the  
249 groups. Specifically, the distributions are more positively skewed in the SICMA  
250 (skewness: V1 = 1.7, A1 = 1.7; kurtosis: V1 = 1.8, A1 = 2.3) than the Pav group  
251 (skewness: V1 = 1.2, A1 = 0.8; kurtosis: V1 = 1.3, A1 = -0.2), with the majority of SICMA  
252 observations consisting of a single response. Indeed, the median response rate in the  
253 SICMA group for both cues was 1, whereas that in the Pav group was 2.7. A Mann-  
254 Whitney test confirmed that rats in the SICMA group made fewer head entries than  
255 those in the Pav group both during V1 ( $U = 222$ ,  $p < 0.0001$ ) and A1 ( $U = 220$ ,  $p < 0.0001$ ).  
256 Such a low response variability in SICMA-trained rats discourages the use of rate of  
257 head entries as a dependent variable in SICMA studies, and confines any conclusions  
258 drawn from group comparisons here to percent responding. In any case, it is worth  
259 noting that we (unpublished) and others (e.g., Takahashi et al., 2013) have found that,  
260 likely due to the physical restraint imposed by the tether, electrode-implanted animals  
261 also express discrimination learning more clearly in percent responding than rate of  
262 head entries in the Pavlovian magazine approach procedure.

263 For the purpose of statistical analyses, the data from each subject was first  
264 averaged across trials in a session, and further collapsed into average responding in  
265 two-session blocks. Analyses of the cues A1/A2 and V1/V2 were conducted separately,

266 as these two subsets of cues differ in both modality and probability of reward, making  
267 comparisons across cue pairs uninformative. Results were analyzed using a mixed-  
268 model linear analysis ANOVA, and Bonferroni-corrected simple-effects analysis to  
269 decompose significant interactions when present. All calculations were conducted in  
270 JAMOVI (Gallucci, 2017; The Jamovi Project, 2019).

271

## 272 2.2. Results and Discussion

273 Overall, SICMA rats completed all trials on 94% of the sessions. The mean session  
274 duration was 73.1 min (SD = 25.1) in the SICMA group and 77.5 min in the Pav group.  
275 The effective mean ITI in the SICMA group (10-s ITI + latency to nose poke after trial-  
276 availability cue onset + 30-s no-initiation trials) was 21 s (SD = 11.8 s).

277

### 278 *2.2.1. Comparison of the temporal dynamics of magazine approach between the groups*

279 Due to task requirements, SICMA rats started each trial with their nose in the noseport  
280 and thus had a constant distance (~8 cm) to travel to enter the adjacent reward  
281 magazine (Fig. 2). In contrast, the distance between rats in the Pav group and the  
282 magazine at the start of each trial could vary. To investigate the impact of such  
283 differences in starting location at cue onset on the topography of conditioned  
284 responding, we calculated the second-by-second percentage of time spent in the  
285 magazine during the visual (Fig. 3, top left panel) and auditory (Fig. 3, top right panel)  
286 cues in the final 2-session block of training. Overall, rats in the SICMA group responded  
287 more to both the visual ( $F_{\text{Grp}}(1,30)=7.91$ ,  $p=0.009$ ) and auditory ( $F_{\text{Grp}}(1,30)=7.43$ ,  
288  $p=0.011$ ) stimuli, compared to rats in the Pav group. This difference between groups

289 emerged across the duration of the cues (visual:  $F_{\text{Grp}^*\text{Sec}}(9,570)=182.57$ ,  $p<0.001$ ;  
290 auditory:  $F_{\text{Grp}^*\text{Sec}}(9,570) = 15.96$ ,  $p<0.001$ ). With regard to the visual stimuli, only  
291 V1(100%) produced differential levels of responding between the two groups  
292 ( $F_{\text{Grp}^*\text{Sec}^*\text{CS}}(9,570)=2.37$ ,  $p=0.012$ ). In the first second following CS onset the groups did  
293 not significantly differ in their response to V1(100%) ( $F(1,82.4)=0.42$ ,  $p\approx 1$ ) or V2(0%)  
294 ( $F(1,82.4)=0.90$ ,  $p\approx 1$ ), but for all subsequent seconds the SICMA group showed  
295 significantly higher levels of responding to V1(100%) than the Pav group  
296 ( $F(1,82.4)=14.40-25.08$ ,  $P_s<0.02$ ). Although this suggests a higher response ceiling in  
297 the SICMA group, it is worth noting that the groups did not differ in their ability to  
298 withhold responding in the presence of V2(0%) ( $F(1,82.4)=0.002-1.04$ ,  $P_s\approx 1$  in all  
299 seconds beyond the first). Thus, the higher response ceiling for V1(100%) in the SICMA  
300 group does not appear to result from an indiscriminate elevation of baseline responding  
301 in these animals.

302 As for the auditory discrimination, no overall between-group differences in  
303 responding to stimuli A1(75%) and A2(25%) were detected in the first 5 s of cue period  
304 ( $F(1,43.3)=3.21-7.91$ ;  $p>0.08$ ). Notably, responding was numerically greater in the Pav  
305 than the SICMA group in the very first second, presumably indicating that some Pav  
306 rats may have been near or even inside the reward magazine at the time of cue onset—  
307 a physical impossibility for SICMA animals. Greater responding to these cues in the  
308 SICMA relative to the Pav group did reach significance in the sixth second, and stayed  
309 significant for the remainder of the auditory cues period ( $F(1,43.3)=10.22-13.41$ ,  
310  $p<0.03$ ). Thus, this result suggests that the SICMA procedure might encourage greater  
311 responding to partially reinforced cues, at least from the auditory modality.

312

### 313 *2.2.2. Comparison of discrimination learning between the groups*

314 To compare discrimination learning between the groups, we analyzed magazine activity  
315 during the cues across the five two-session blocks of training (Fig. 3, bottom panels).  
316 Following Holland (1977), we focused our analysis on the last 5 s of CS period, where a  
317 more stable readout of magazine activity can be obtained (Fig. 3, top panels). The  
318 results, shown in the bottom panels of Fig. 3, confirmed that rats across the two groups  
319 solved both the visual ( $F_{CS}(1,270)=322.67$ ,  $p<0.001$ ) and auditory discriminations  
320 ( $F_{CS}(1,270)=75.005$   $p<0.001$ ).

321 Unsurprisingly, the solution of the visual discrimination emerged in both group as  
322 training progressed ( $F_{Bik*CS}(4,270)=14.87$ ;  $p<0.001$ ; Fig. 3, bottom left panel). More  
323 importantly, this discrimination was solved more readily by the SICMA than the Pav  
324 group. ( $F_{Grp*Bik*CS}(4,270)=2.89$ ,  $p=0.023$ ). Simple effects analyses revealed that the  
325 SICMA group showed significant evidence of discrimination learning between the visual  
326 cues from session block 2 onwards ( $F(1,270)=45.18-151.67$ ,  $p<0.015$ ). In contrast, the  
327 Pavlovian group only showed significant evidence of discrimination learning starting on  
328 session blocks 4 and 5 ( $F(1,270)=23.41-26.15$ ,  $p<0.015$ ). Additionally, there was a  
329 significant difference between the groups in overall level of responding on the first block  
330 of training ( $F(1,53.6)=53.86$ ,  $p<0.015$ ).

331 As expected, the auditory discrimination (Fig. 3, bottom right panel) similarly  
332 emerged over the course of training in both groups ( $F_{Bik*CS}(4,270)=3.910$ ,  $p=0.004$ ).  
333 Simple effects analysis showed that, combined, both groups responded significantly  
334 more to A1(75%) than A2(25%) from the second session block onwards

335 (F(1,270)=13.132-34.313,  $p < 0.01$ ). Due to the lack of a significant three-factor  
336 interaction, it is safe to interpret this finding as indicating that both groups solved the  
337 auditory discrimination by the second block of training and did not significantly differ  
338 from each other in their ability to discriminate the cues. There was, however, a  
339 significant difference between the groups in baseline levels of responding  
340  $F_{\text{Grp}}(1,30)=15.229$ ,  $p < 0.001$ ), which changed over the course of training  
341 ( $F_{\text{Blk} \times \text{Grp}}(4,270)=6.374$ ,  $p < 0.001$ ). Simple effects analysis of this interaction showed that  
342 the groups significantly differed in their overall level of responding in blocks 1,4 and 5  
343 ( $F(1,47.1)=29.73$ ,  $p < 0.01$ ;  $F(1,47.1)=9.72$ ,  $p=0.03$  and  $F(1,47.1)=15.86$ ,  $P_s < 0.01$ ,  
344 respectively), but not in blocks 2 or 3 ( $F(1,47.1)=5.30-6.46$ ,  $P_s > 0.14$ ). This baseline  
345 difference aside, the results of Experiment 1 show that, despite the short ITI, rats  
346 trained with the SICMA procedure showed no worse (and if anything, better)  
347 discrimination performance than rats trained with the standard Pavlovian magazine  
348 approach procedure.

### 349 **3. Experiment 2 – Comparison of SICMA and yoked Pavlovian magazine-approach** 350 **groups**

351 This experiment aimed to provide a more direct comparison between the SICMA  
352 procedure and the Pavlovian magazine-approach method by imposing identical training  
353 conditions except for the requirement self-initiation. To this end, a yoked procedure was  
354 used in which animals in the Pavlovian group (labeled Yoked) received the exact same  
355 sequence of experimental events and, critically, at the same time, as their self-initiating  
356 counterparts in the SICMA group, ensuring an equal number of equally spaced trials.

357 3.1. Materials and Methods

358 *3.1.1. Animals & Apparatus*

359 Eight male and eight female adult Long-Evans rats bred at Brooklyn College from rats of  
360 Charles River descent were used (Charles River Laboratories). At the start of the  
361 experiment, all rats were approximately 90 (+/- 7) days old and their weights ranged  
362 between 239 and 253 g for females and 301 and 334 g for males. They were kept under  
363 the same husbandry conditions as described in Experiment 1. Experimental sessions  
364 were conducted between 3-5 hours after the onset of the light phase of the cycle. The  
365 apparatus used was that described in Experiment 1.

366

367 *3.1.2. Procedure*

368 Animals were randomly assigned to two groups, labeled SICMA and Yoked (8 rats per  
369 group, gender balanced). In the SICMA group, magazine training, shaping and  
370 discrimination training procedures were identical to those used in Experiment 1.  
371 Following magazine training, rats in the Yoked group were each paired with a master rat  
372 in the SICMA group. This ensured that each rat in the Yoked group received the same  
373 exact sequence of events and at the same time as it was being experienced by its  
374 master rat in the SICMA group. This included noseport light illumination at the start of  
375 each trial-availability period in the SICMA group. Thus, the only difference between the  
376 two groups was that the yoked rats had no behavioral control over trial initiation. The  
377 results were analyzed with the same statistical tests used in Experiment 1.

378

379 3.2. Results and Discussion



380 SICMA rats completed all trials on 96% of the sessions (idem, of course, in the yoked  
381 rats). The session duration in the groups was 53.8 min on average, with a SD of 11.5  
382 min. The effective mean ITI in the SICMA group (10-s ITI + latency to nose poke after  
383 trial-availability cue onset + no-initiation trials) was 20.4 s (SD = 7.3 s).

384

### 385 *3.2.1. Comparison of the temporal dynamics of magazine approach between the groups*

386 To examine potential differences in response topography due to between-group  
387 differences in the rats' distance to the reward magazine at cue onset, once again we  
388 analyzed the temporal profile of magazine approach across the 10 s of CS presentation,  
389 focusing on the final two-session block of training (Fig. 4, top panels). Overall, rats  
390 showed changes in responding over the 10 s for both the visual ( $F_{\text{Sec}}(9,266)=11.239$ ,  
391  $p<0.001$ ) and auditory ( $F_{\text{Sec}}(9,266)=8.117$ ,  $p<0.001$ ) discriminations. Interestingly, this  
392 effect of Second into stimulus presentation interacted with the Group factor for both  
393 visual ( $F_{\text{Grp*Sec}}(9,266)=5.192$ ,  $p<0.001$ ) and auditory modalities ( $F_{\text{Grp*Sec}}(9,266)=3.212$ ,  
394  $p=0.001$ ). Indeed, the top panels of Fig. 4 show that rats in the SICMA group  
395 progressively increased responding after the first second of the better predictor in each  
396 discrimination, whereas rats in the Yoked group were consistent across its duration, an  
397 observation that was confirmed by simple effects analysis for both the visual  
398 ( $F_{\text{SICMA}}(9,266)=15.07$   $p<0.004$  and  $F_{\text{Yoked}}(9,266)=1.36$   $p=0.816$ ) and auditory modalities  
399 ( $F_{\text{SICMA}}(9,266)=10.345$ ,  $p<0.004$  and  $F_{\text{Yoked}}(9,266)=0.984$ ,  $p\approx 1$ ). Thus, this finding  
400 indicate a greater dynamic range of responding for SICMA than Pav subjects under the  
401 present training conditions (i.e., short ITI). The SICMA group responded less than the  
402 Yoked group in the first second of both discriminations, although this trend was not

403 significant. Once again, this suggests that some of the Yoked animals were immediately  
404 adjacent to or inside the reward magazine at the time of cue onset, as would be  
405 expected given the short ITI.

406

### 407 *3.2.2. Comparison of discrimination learning between the groups*

408 As in Experiment 1, to determine if and when the groups solved the two discriminations  
409 across training, we analyzed magazine activity across all five two-session blocks,  
410 focusing on the last 5 s period of CS presentation (Fig. 4, bottom panels). A main effect  
411 of Stimulus was significant in both modality discriminations (Visual:  $F_{CS}(1,126)=26.697$ ,  
412  $p<0.001$ ; Auditory:  $F_{CS}(1,126) = 29.59$ ,  $p<0.001$ ), indicating that all rats considered  
413 together were able to discriminate between the cues as training progressed.  
414 Furthermore, a main effect of Session block was likewise significant (Visual:  
415  $F_{Blk}(4,126)=3.226$ ;  $p=0.015$ ; Auditory:  $F_{Blk}(4,126)=8.4560$ ,  $p<0.001$ ), confirming that, as  
416 the bottom panels of Fig. 4 show, the discriminations were solved by withholding  
417 responding over the course of training to the less predictive CSs (V2 and A2) without  
418 increasing responding to the more predictive ones (V1 and A1).

419 Critically, as evident in the bottom panels of Fig. 4, the SICMA group showed  
420 better discrimination learning than the Yoked group, and this was true of the visual  
421 ( $F_{Grp*CS}(1,126)=8.992$ ,  $p=0.003$ ) and auditory ( $F_{Grp*CS}(1,126)=4.230$ ,  $p=0.042$ )  
422 modalities. Indeed, the visual discrimination achieved statistical significance in the  
423 SICMA ( $F(1,126)=33.34$ ,  $p<0.001$ ), but not the Yoked group ( $F(1,126)=2.35$ ;  $p=0.128$ ).  
424 On the other hand, both the SICMA and Yoked groups solved the auditory  
425 discrimination to a significant degree ( $F_{SICMA}(1,126)=28.10$ ,  $p<0.002$  and

426  $F_{\text{Yoked}}(1,126)=5.72$ ,  $p=0.036$ , respectively), although the SICMA animals solved this  
427 discrimination with a larger effect size (95% confidence interval of difference in percent  
428 responding: 4.662-10.22) than the Yoked rats did (0.580-6.13). Thus, discriminative  
429 performance in the Yoked group achieved significance in the case of the auditory, but  
430 not the visual discrimination, despite the latter being simpler in terms of the reward  
431 probabilities involved (100% vs 0% as opposed to 75% vs. 25% in the auditory case).  
432 This may simply reflect the superior perceptual discriminability of the auditory relative to  
433 the visual cues we used. Taken together, the results in the Yoked group confirm the  
434 deleterious effects of a short ITI in the conditioned magazine-approach preparation  
435 (e.g., Lattal, 1999; Holland, 2000)., and highlight the risk associated with shortening the  
436 ITI in neural recording studies using Pavlovian conditioning. Crucially, such deleterious  
437 effects were not observed in the SICMA group despite having an equally short ITI, the  
438 implications of which are considered in the General Discussion.

439

#### 440 **4. Experiment 3 – Does conditioned responding to self-initiated cues transfer** 441 **when the cues are delivered in the standard Pavlovian fashion?**

442 A notable difference between SICMA and the standard Pavlovian procedure is that  
443 SICMA requires shaping an instrumental nose-poke response at the noseport prior to  
444 the start of discrimination training. This raises the question of whether SICMA-trained  
445 rats come to treat the cues as Pavlovian CSs (i.e., cues that evoke Pavlovian  
446 conditioned approach responses) or rather as discriminative stimuli that inform the  
447 animal of when to complete an instrumental action sequence consisting of a nose poke  
448 followed by magazine approach. Although we would argue that neither associative

449 structure would detract from the advantages of SICMA for neural recording, one  
450 particular scenario would render this procedure less useful. If during shaping rats  
451 acquire a noseport poke→magazine approach action sequence, they could conceivably  
452 ignore reinforced CSs and learn only about cues that signal the omission of  
453 reinforcement. If this is the case, then reinforced cues trained with SICMA should evoke  
454 little magazine approach when delivered in a Pavlovian fashion (i.e., without self-  
455 initiation). In contrast, if reinforced cues trained with SICMA are attended to and learned  
456 about, such a transfer should be relatively seamless. Experiment 3 allows for the  
457 dissociation of these two possibilities.

458

#### 459 4.1. Materials and Methods

##### 460 *4.1.1. Animals & Apparatus*

461 Four male and four female adult Long-Evans rats were used, bred at Brooklyn College  
462 from rats of Charles River descent. At the start of the experiment, all rats were  
463 approximately 90 (+/- 7) days old and their weights ranged between 242 and 257 g for  
464 females and 311 and 345 g for males. Husbandry and apparatus details were identical  
465 to those reported in the previous experiments.

466

##### 467 *4.1.2. Procedure*

468 Magazine training, shaping and discrimination training procedures were identical to  
469 those used in the SICMA group of Experiment 1, except that animals received 20  
470 sessions. The day after the last SICMA session, a single Pavlovian transfer session was  
471 conducted in which the rats were presented with the same discrimination. The

472 procedural details in this test session were identical to those used in the Pav group of  
473 Experiment 2.

474

#### 475 4.2. Results and Discussion

476 Trials were averaged into 2-trials blocks. We used a series of uncorrected within-  
477 subjects t-test to determine if performance in the Pavlovian transfer session was  
478 significantly different from that at final 2-trial block of SICMA training. We chose not to  
479 correct these t-test for multiple comparisons, as in this case we hypothesized that these  
480 conditions would not produce significant differences. As can be seen in Fig. 5, rats'  
481 conditioned magazine activity to visual (top panel) and auditory (bottom panel) cues  
482 was virtually identical in the last 2-trial block of SICMA training and all-trial blocks of the  
483 Pavlovian transfer session. To ensure that these similarities were not due to rapid  
484 within-session acquisition, we focused our analysis on the first 2-trial block of the  
485 Pavlovian session. For the visual discrimination, t-tests found no significant differences  
486 in responding to V1 ( $t(7)=1.42$   $p=0.196$ ) or V2 ( $t(7)=0.19$   $p=0.857$ ), and these results  
487 were mirrored for the auditory cues ( $t(7)=2.08$ ,  $p=0.075$  for A1 and  $t(7)=-0.404$ ,  $p=0.698$   
488 for A2). Thus, even under conditions favorable to detecting a difference (a series of  
489 uncorrected t-tests), the results confirm that the predictive significance of the cues was  
490 preserved when the cues were subsequently presented without self-initiation to animals  
491 that had never previously received Pavlovian training. This is inconsistent with the  
492 hypothesis that SICMA training discourages rats from attending to and learning about  
493 reinforced cues, at least when the latter are embedded in a discrimination.

494

## 495 **5. General discussion**

496 Probing the neural mechanisms of cue-reward learning is often hindered by the difficulty  
497 in adapting extant Pavlovian preparations to the parametric requirements of neural  
498 recording. In this article, we introduced SICMA, a self-initiated variant of the Pavlovian  
499 magazine-approach procedure designed to empower the electrophysiologist working  
500 with rodents. Unlike its Pavlovian predecessor, SICMA allows extensive sampling of  
501 multiple trial types in a short space of time, leveraging the experimenter's ability to  
502 detect real patterns in the neural data without compromising learning.

503 A further advantage of SICMA for neural recording is that it guarantees that at  
504 the onset of each CS the animal is in the same location within the conditioning chamber.  
505 This will help reduce trial-to-trial variability in neuronal responses caused by location-  
506 dependent changes in the perception of the stimuli and/or by the juxtaposed encoding  
507 of spatial and cue-related information. In addition, SICMA ensures that at the onset of  
508 each CS the animal is engaged and thus more likely to consistently garner task-relevant  
509 attentional resources that would likely fluctuate across trials over the course of a long  
510 Pavlovian session. Indeed, a disadvantage of the standard magazine-approach  
511 procedure for neural recording is the possibility that the animal might become oblivious  
512 of the CSs as they continue to be presented.

513 A higher level of engagement in SICMA might go some way to explaining the  
514 superior performance observed in this condition relative to the yoked Pavlovian control.  
515 However, other explanations should be considered, particularly to account for SICMA's  
516 imperviousness to the detrimental effects of massed trials on learning so typical of  
517 Pavlovian conditioning preparations. The latter effects are commonly attributed to

518 lessened extinction of the context due to the high frequency of reinforcement, which will  
519 enhance the context's ability to compete with discrete CSs for behavioral control (e.g.,  
520 Rescorla, Durlach & Grau, 1985). By making trial initiation contingent upon an  
521 instrumental response (e.g. poking in the noseport to turn on the CSs), the role of the  
522 context as a predictor of reward might drastically diminish in SICMA. In addition,  
523 deleterious memory-interference effects might have less impact on learning in SICMA  
524 than in the yoked Pavlovian group. For instance, any proactive interference resulting  
525 from lingering short-term memory traces carrying over to the next trial would be  
526 attenuated in SICMA if the trial-initiating response can *reset* the short-term memory  
527 buffer (Dunnett & Martel, 1990). Alternatively—or additionally—agency over trial-  
528 initiation might reduce retroactive interference of each trial with rehearsal of the  
529 preceding trial by removing any element of surprise that trial presentation has when  
530 delivered in a Pavlovian fashion with a variable ITI. This would place SICMA rats at an  
531 advantage over yoked ones in light of evidence that a surprising event presented shortly  
532 after a trial can disrupt learning on that trial (Wagner, Rudy & Whitlow, 1973). Future  
533 investigations of these mechanisms will not only inform the use of SICMA, but more  
534 broadly, shed light on the role of agency in predictive learning.

535         While the current procedure offers a series of advantages for neural recording, it  
536 also comes with some downsides. Notably, the self-initiation aspect of the procedure  
537 makes it in principle difficult to apply to the study of aversive conditioning. Even if an  
538 aversive component were superimposed on the appetitive task, the number of aversive  
539 trials would necessarily have to be relatively small if the animal is not to be discouraged  
540 from performing altogether—in all likelihood small enough to represent no advantage

541 over extant aversive procedures. Furthermore, giving the animal control over trial  
542 initiation requires a minimum, nonzero overall rate of reinforcement in order to maintain  
543 the animal's motivation to perform. Extensive pilot work in our laboratory has revealed  
544 that rats will perform in SICMA for ~100 trials at a 25% overall reward rate, and it is  
545 possible that an even lower rate might support behavior in well-trained animals. That  
546 said, it is still the case that SICMA will not be the procedure of choice for studies  
547 involving long blocks of nonreinforced trials presented consecutively and with no  
548 intervening reinforced trials. Lastly, as hinted above, SICMA will also be of little use to  
549 researchers investigating the neural bases of contextual conditioning, as in SICMA the  
550 context is rendered unpredictable of reward. Interestingly, eliminating the contribution of  
551 contextual conditioning to cue-evoked conditioned responding provides a less  
552 ambiguous readout of the cue's predictive significance (i.e., uncontaminated by context-  
553 elicited conditioned responding), which will be advantageous to researchers specifically  
554 interested in cue-reward learning.

555         To the extent SICMA and standard Pavlovian training might engage different  
556 cognitive processes (e.g., heightened attention to the task, diminished competition by  
557 the context, etc.), one must exert caution when generalizing the results from SICMA  
558 studies to Pavlovian settings. The smooth transfer of discriminative performance across  
559 the SICMA and Pavlovian phases of Exp. 3, however, tentatively argues for a common  
560 discrimination-learning mechanism that informs decision-making under different  
561 behavioral requirements. It is upon the neural implementation of that mechanism that  
562 SICMA can shed light where Pavlovian preparations fall short. Thus, we anticipate the  
563 procedure will be particularly useful in neural recording studies using complex, within-



564 subject discrimination designs (e.g., four trial types or more), such as those typical of  
565 stimulus selection, nonlinear discriminations, categorization and rule learning studies.

566 To conclude, we would argue that a more general limitation of appetitive  
567 Pavlovian procedures is that the animal's role is restricted to that of an opportunistic  
568 agent aiming to exploit environmental contingencies beyond its control. By granting the  
569 animal agency to seek out cues potentially predictive of reward, the SICMA procedure  
570 offers a complementary, also ecologically-relevant way to model appetitive learning.

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#### 578 **CRedit author statement**

579 **Ingrid Reverte:** Conceptualization, Methodology, Software, Investigation, Data  
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585 Software. **Mihaela Iordanova:** Writing- Reviewing and Editing. **Guillem R. Esber:**

586 Conceptualization, Project Administration, Methodology, Supervision, Writing- Original  
587 draft preparation, Writing- Reviewing and Editing, Funding acquisition.

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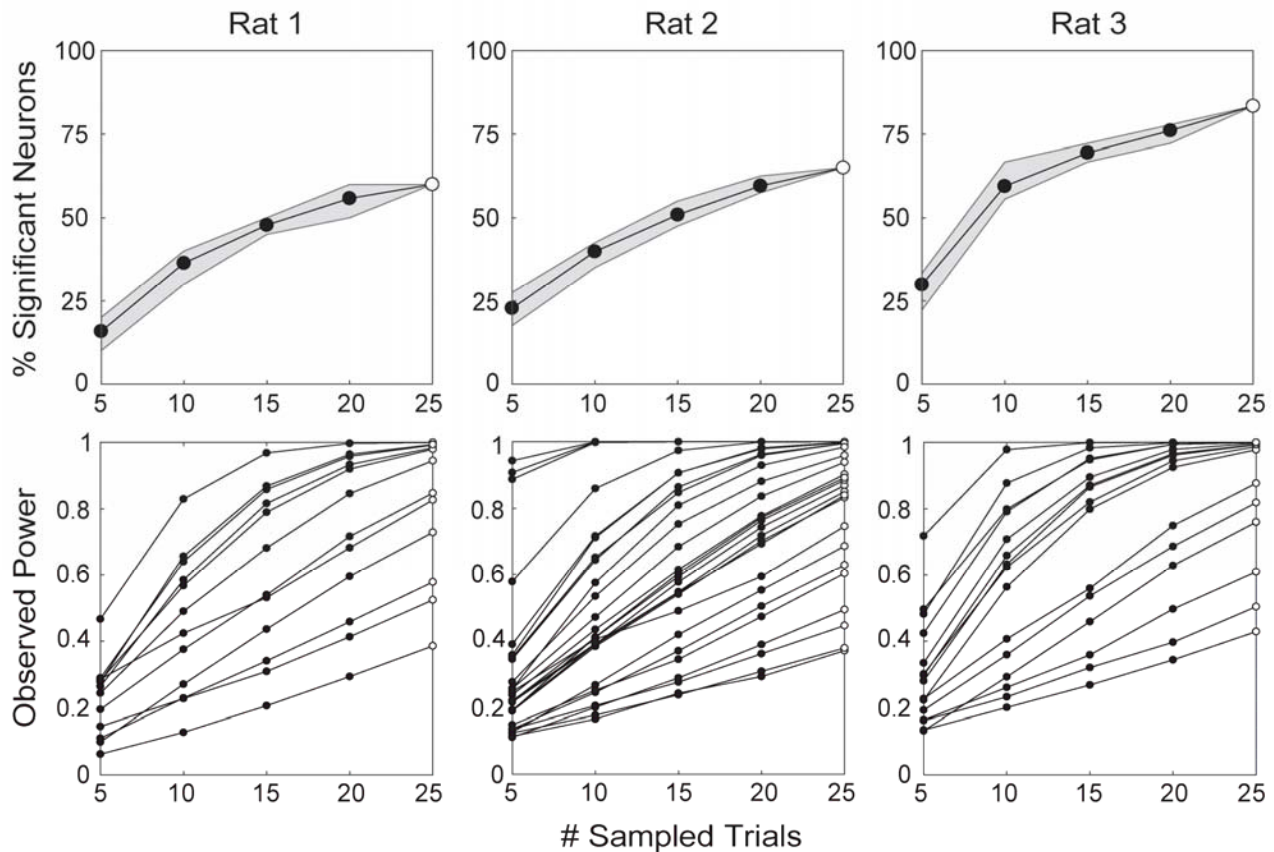
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735 **Figures**

736



737

738 *Figure 1.* Results of a series of bootstrap analyses demonstrating the importance of  
739 large trial counts for investigating the neural correlates of predictive learning (see  
740 Supplemental Materials). All analyses were conducted using spike rates during CS  
741 period of neurons recorded in the orbitofrontal cortex of three rats (columns) on the final  
742 session of discrimination training of the form V1+, V2-, V1V2-, where V1 and V2  
743 represent two 10-s visual cues, while the “+” and “-” symbols represent reinforcement  
744 and non-reinforcement, respectively. Each trial type was presented 25 times in a  
745 session, adding up to a total of 75 trials. The top panels show the percentage of  
746 neurons that significantly discriminated between reinforced and non-reinforced cues as

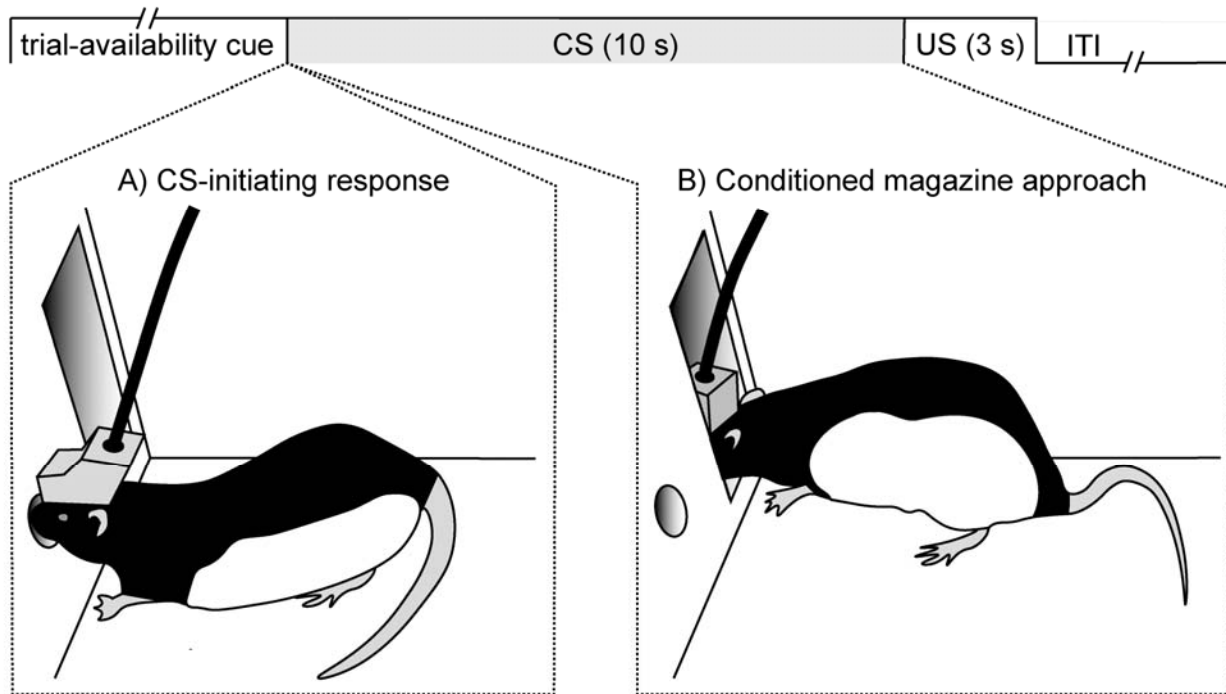
747 a function of the number of trials sampled, as identified by a one-way ANOVA ( $p < 0.05$ ).

748 Shaded areas represent the 75% and 25% quartiles of the bootstrap iterations. The

749 bottom panels depict the mean observed statistical power in the same ANOVA for each

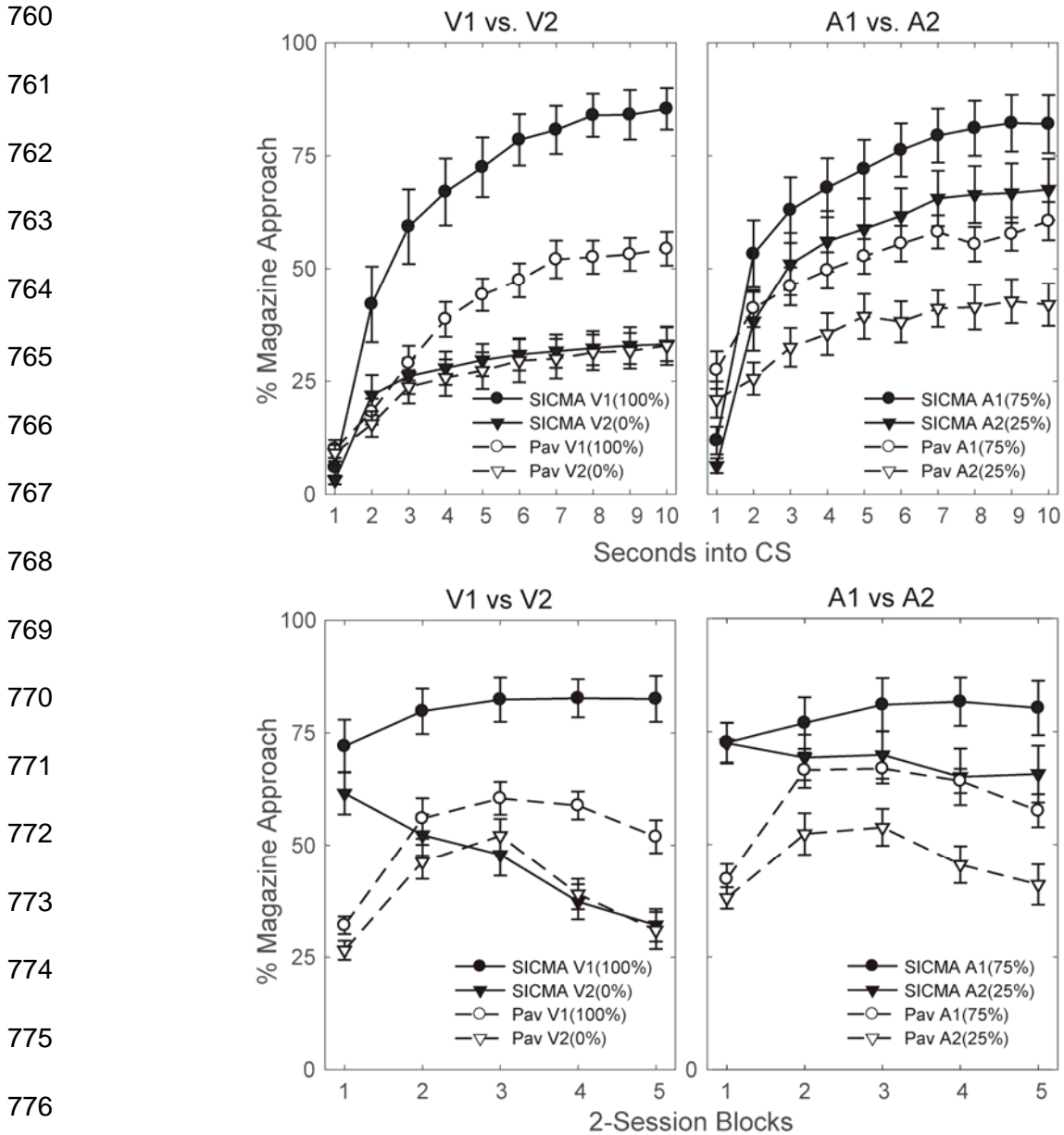
750 neuron recorded, also plotted as a function of the number of trials sampled. Open

751 circles represent the actual results when all 25 trials presented were included.



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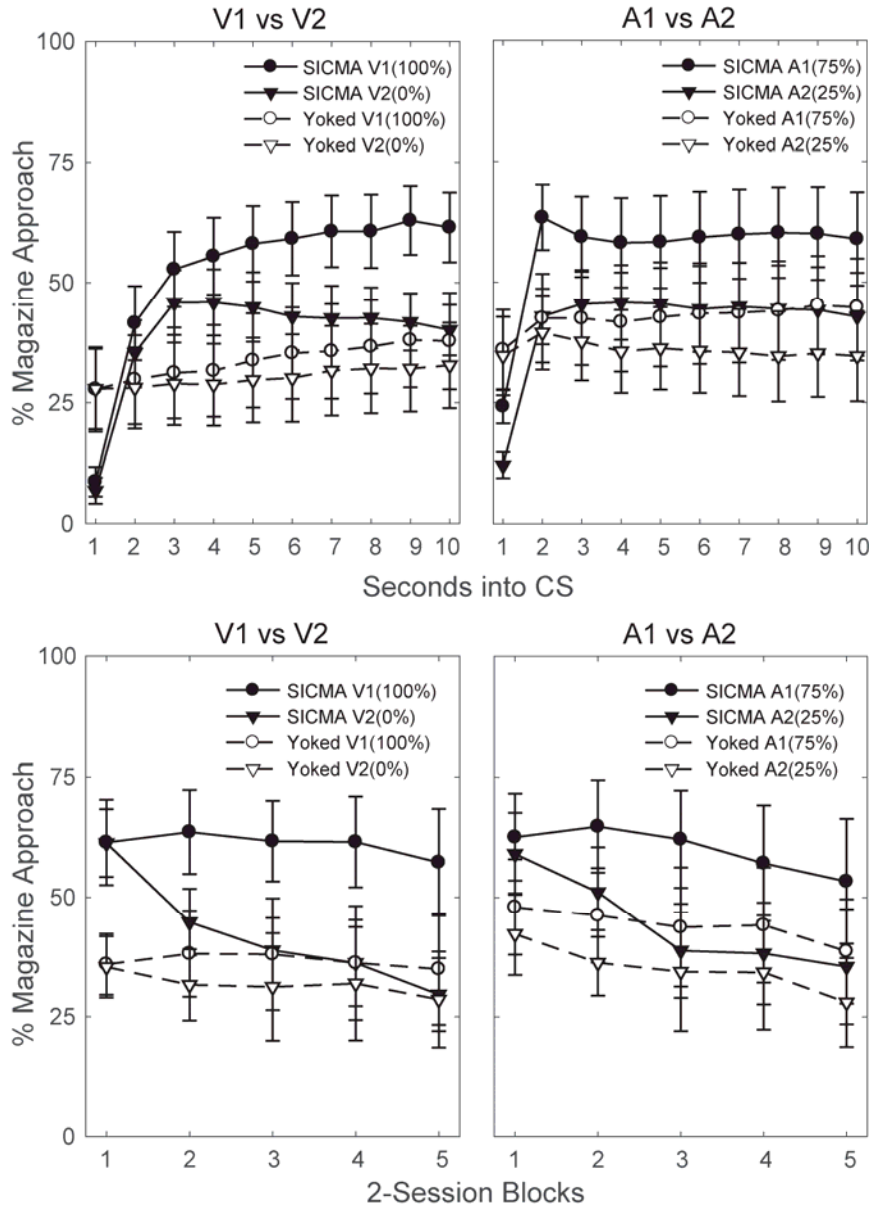
753 *Figure 2.* Trial schematic of the SICMA procedure. A light cue inside the noseport  
754 signals trial availability for a maximum of 20 s, during which the animal can respond at  
755 the noseport (panel A) to turn on one of several possible CSs. During the 10-s CS, the  
756 animal may perform anticipatory approach responses in the reward magazine (panel  
757 B)—just as in the standard magazine-approach procedure. On reinforced trials, a  
758 reward (US) is delivered at the end of the CS, followed by an average intertrial interval  
759 of 10 s.



778 *Figure 3.* Comparison of conditioned magazine-approach performance in a visual (left  
 779 panels) and auditory (right panels) discrimination between the SICMA and Pavlovian  
 780 groups. The top panels show the time course of responding to the CSs in the final 2-  
 781 session block of training, expressed as the mean percentage of time the rats spent in  
 782 the magazine in each of the 10 s of cue presentation. The bottom panels show the

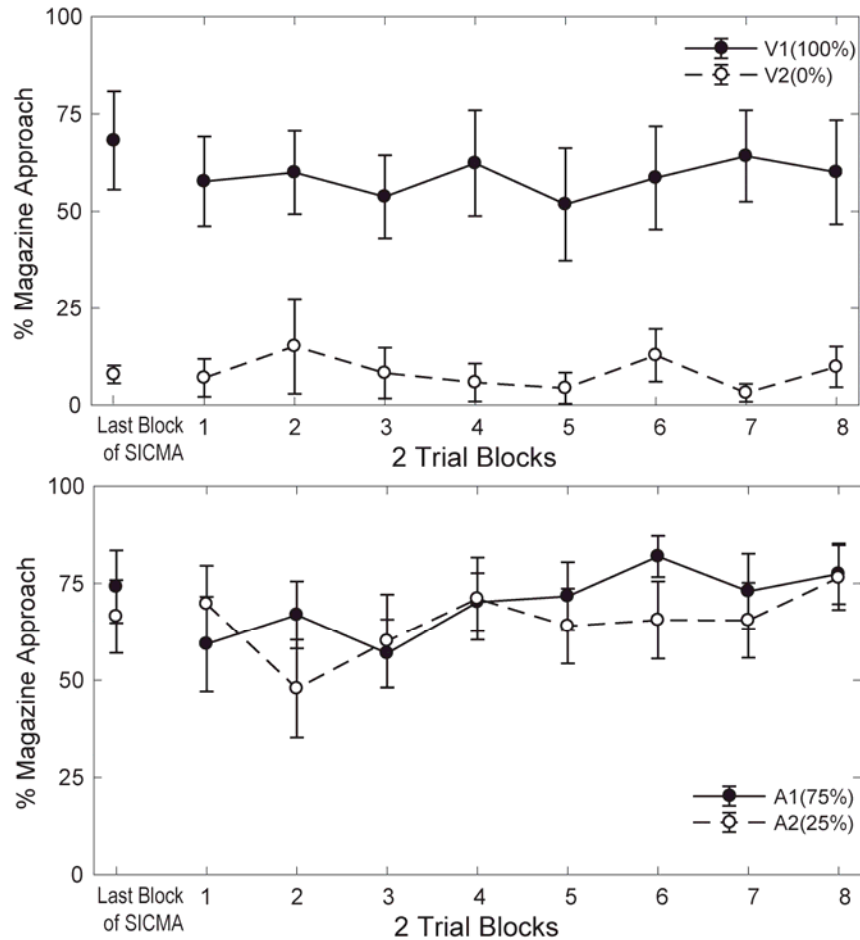
783 mean percentage of time the rats spent in the magazine during the 10-s CSs across the  
784 five 2-session blocks of discrimination training. Error bars represent the standard error  
785 of the mean (SEM).

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803 *Figure 4.* Comparison of conditioned magazine-approach responding in a visual (left  
804 panels) and auditory (right panels) discrimination between the SICMA and Yoked  
805 Pavlovian groups. The top panels depict the time course of responding to the CSs in the  
806 final 2-session block of training, expressed as the mean percentage of time the rats  
807 spent in the magazine in each of the 10 s of cue presentation. The bottom panels show  
808 the mean percentage of time the rats spent in the magazine during the last 5 s of CS

809 period across the five 2-session blocks of discrimination training. Error bars represent  
810 the standard error of the mean (SEM).



811

812 *Figure 5.* Results of a SICMA-to-Pavlovian transfer test. The same visual and auditory  
813 discriminations used in Experiments 1 and 2 were first trained in the SICMA procedure  
814 and then tested in a Pavlovian fashion (i.e., without self-initiation). The figure provides a  
815 comparison of conditioned magazine-approach performance between the last 2-trial  
816 block of the final SICMA session and all 2-trial blocks of the subsequent Pavlovian  
817 session. Only data from the last 5 s of cue presentation was considered. Error bars  
818 represent the within-subject SEM.