

Incidental Encoding and Retrieval of Episodic-Like Memory in Rats: A Novel Behavioral Task

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Abstract

There is a pressing need for an ecologically relevant behavioral task that would enable the study of elementary aspects of episodic memory. In everyday life, episodic memories are acquired incidentally in a single-encounter fashion and are composed of sub-events separated by time gaps. We designed a behavioral task called One-Trial Trace Escape Reaction (OTTER), in which rats incidentally associated two temporally discontinuous stimuli. In OTTER, rats associate a neutral acoustic cue (conditioned stimulus, CS) with an aversive stimulus (unconditioned stimulus, US) which occurs two seconds later: we call this sequence CS-2s-US. In the first few sessions, rats are habituated to two similar environmental contexts (A and B); each context consists of interconnected dark and light sub-areas. Next, in the pairing session, rats experience CS-2s-US in the dark sub-area of one of the environmental contexts (either A or B). The US is terminated immediately after a rat escapes into the light sub-area. During the recall session 24 hours later, rats are presented with only the CS in the alternate environmental context (B or A) and their behavioral response is observed. Our results show that 50% of handled rats and 14% of non-handled rats responded to the CS by escaping to the light sub-area although they experienced only a single CS-2s-US pairing. The capacity to acquire a CS-2s-US association in a single CS-2s-US pairing indicates that rodents are able to form incidental temporal associations. The OTTER behavioral task offers a flexible high throughput tool to study memories acquired incidentally after a single experience.

Results And Discussion

Episodic memory is the ability to retain and recall knowledge of personally experienced past events[1]. These events are often composed of successive sub-events separated by time gaps and might be experienced as a single memory[2]. The ability to form associations between events, known as temporal binding, is likely an essential prerequisite for the formation of more complex episodic memories[3]. Another aspect of episodic memories is that they are acquired incidentally, i.e., we remember events we did not intend to memorize. In an experimental setup, incidental memory can be tested in situations when subjects are not aware that they will be tested on recall[4–6]. We believe that the encoding of episodic-like memory should not require conditioning or pretraining and evidence suggests that mechanisms of incidental encoding might differ from encoding with intent[7–10].

In order to ultimately understand the neural mechanism of episodic memory it is vital to first elucidate the neural mechanisms of encoding and retrieval of temporally bound sub-events. The first step towards this goal is to develop a valid and reliable behavioral task. Our goal was to develop a simple temporal binding task with a clear behavioral response and with a balanced ratio of success. We focused on a one-trial design to ensure that memory was encoded incidentally. To achieve this, we took advantage of two natural behavioral tendencies of rodents. Firstly, rodents avoid brightly lit environments[11] and secondly, they actively escape from an immediate threat[12, 13].

Our task, which we named One-Trial Trace Escape Reaction (OTTER), consisted of three stages: habituation, pairing, and recall (Figure 1A–C). The purpose of habituation is to familiarize rats with a

novel environment and to reduce their exploratory activity. Rats were habituated to two similarly constructed environmental contexts; context A was oval-shaped while context B was slightly larger and rectangular-shaped, see (Figure 1A). Both contexts consisted of one dark and one light sub-area, separated by a partition with a rectangular opening. This design allowed us to exploit the natural tendency of rodents to avoid bright light. It should be noted that even when rats are free to move between both sub-areas, they strongly prefer the dark sub-area. To olfactorily diversify both contexts, context A was cleaned with alcohol-based wash, while context B was cleaned using vinegar-based wash.

During the pairing session, rats experience two novel stimuli separated by a time gap, see (Figure 1B). A rat is first allowed to explore the apparatus of context A or B exactly as it would during the habituation sessions. When the rat settles in the dark sub-area, it is presented with an acoustic cue (the conditioned stimulus CS, of three second duration). Two seconds after the CS is terminated the rat receives an electric shock to its feet (unconditioned stimulus, US). The US is terminated immediately after the rat escapes to the light sub-area. This is the opportunity for the rat to incidentally associate the CS with US (CS-2s-US) and learn that escape to the light-sub area provides safety from the US.

Association between the CS and US is tested during the recall session 24 hours later (Figure 1C). Unlike in traditional active avoidance tasks, the recall in OTTER is tested in a different environmental context. Testing the recall in a different environmental context renders the association between the US and environmental context irrelevant, therefore fear-related behavioral responses are only attributable to the association between the CS and US. At the beginning of the recall session, each rat was placed in the alternate environmental context from the one used in the pairing session (B or A). If at least 15 minutes elapsed and the rat rested in the dark sub-area, the CS was delivered, and the reaction of the rat was observed. There are two possible reactions: either the rat escapes into the light sub-area ('responder'), or it remains in the dark sub-area ('non-responder').

We evaluated the effect of animal handling on OTTER performance using Wistar rats. The first group of rats was handled for a week prior to the experiment while the second group experienced only standard manipulation (non-handled). During the 15-minute habituation sessions in environmental contexts A and B, both groups of rats preferred the dark sub-area in both contexts (Figure 2A–E). Despite the difference in shape, scent and area size of environmental contexts A and B, there was no notable difference in the number of transfers (Figure 2F–J) or in time spent in the light sub-area between the handled and non-handled rats (Figure 3).

During the pairing session, all rats transferred to the light compartment to escape the US. The average time to escape was 4.4 ± 3 seconds in handled and 4.9 ± 3.5 seconds in non-handled rats (SEM, 95% confidence level). Five animals (four handled and one non-handled) received only the CS and served as controls.

The rats' responses to the CS during the recall session differed based on handling treatment: 50% handled rats and (14%) of the non-handled rats escaped within 10 seconds of the start of CS (Figure 4A). This result highlights the importance of animal handling on performance in cognitively demanding tasks.

The average time to escape was 4.9 ± 3.3 seconds in handled rats (SEM, 95% confidence level) and 3 seconds in the non-handled rat (Figure 4B). None of the control rats escaped into the light sub-area in a response to the CS.

It is evident, that OTTER is designed so that a single event is sufficient for an animal to acquire knowledge of a contingency without a need for pre-training or any kind of 'priming' the animal that a behaviorally relevant event can be anticipated. The pre-training in existing rodent paradigms can be twofold; firstly, shaping the desired response behavior, or secondly, repeated exposure to stimuli. Both types of pretraining create an expectancy of contingency. For example, in trace fear conditioning the CS-trace-US is presented more than once[14, 15]. Although it is possible that rodents in trace fear conditioning associate CS with US even after a single CS-trace-US exposure, there is no observable variable which could indicate the acquired knowledge. Freezing is an outcome measured in trace fear conditioning and similar tasks, it is a response associated with an absence of an escape route[16], and might indicate behavioral despair as no action can avert the stressor. In this context, it is possible that freezing behavior emerges only after repeated CS-trace-US presentation. From this perspective, OTTER bypasses the potentially non-specific freezing response because a single pairing session results in a clear avoidance response during the recall session in rats.

In OTTER, active avoidance behavior offers an unambiguous binary measure of recall, so that each animal can be confidently singled out as a 'responder' or 'non-responder'. In contrast, fear conditioning tests and novel object recognition tasks use a continuous variable as an indicator of learning, such as freezing or a duration of exploration. When a continuous variable is used to classify animals into discrete groups then setting a threshold is needed. Setting the threshold value might be difficult; in addition, it is not clear how confident we can be about the classification of animals that are just around the threshold values. Therefore, using a continuous outcome variable as a basis for classification might provide unclear results. Rodent tasks with unambiguous output variables usually involve 'declaring' the knowledge by entering a correct place or pressing a correct button[5, 17]. However, in rodents, extensive training is required since declarative behavior (usually approach behavior) must be shaped by the experimenter. The need of extensive training to achieve declarative behavior precludes the study of incidentally acquired memories. In OTTER, the response is binary, and the declarative behavior is acquired without a need for pretraining.

However, to be able to ascribe the escape reaction to the CS-2s-US association, it is essential that we first establish an invariant baseline behavior – the animal has to stay in the dark sub-area most of the time on its own accord. To this aim, we firstly explored the influence of rat strain on behavior within the same environmental context and discovered that Wistar rats displayed the most suitable behavior for OTTER. Due to their increased light sensitivity, Wistar rats naturally spent most of their time in the dark sub-area and transferred less frequently to the light sub-area when compared to Sprague-Dawley and Long-Evans rats (Supplementary Fig. S1A-B online). Secondly, we changed the size and shape of the opening in the partition between compartments which further reduced time Wistar rats spent in the light sub-area (Supplementary Fig. S2A–B online). By implementing these measures rats seldom spontaneously move

to the light sub-area, hence it is very likely that when an animal 'responds' to the CS, it does so because it remembers the CS-2s-US association and not because of a random exploration.

Notably, OTTER displays no 'ceiling effect' and offers a good balance between 'responders' and 'non-responders' in the handled group (approximately 1:1). Comparison between 'responders' and 'non-responders' may be valuable when tracing neural changes in early acquisition of episodic-like memory. The ratio of 'responders' and 'non-responders' is strongly shifted towards 'non-responders' in non-handled animals (86% of rats were 'non-responders'). It is possible that the presence of an experimenter and/or manipulation stressed the rats. Acute stress, e.g., by a predator, was previously shown to impair consolidation and retrieval of hippocampus-dependent memories[18] which could explain the decreased performance in non-handled rats.

The OTTER task is very flexible. As long as the general principle of OTTER is adhered to, namely, controlling animal behavior by balancing conflicting species-specific behavioral tendencies, OTTER can be embodied by different physical instances. We are in the process of developing a second variant of OTTER with the working title 'ice-OTTER.' In 'ice-OTTER,' the invariant behavior in rats and mice is achieved by utilizing their preference for warmth, which results in the avoidance of the cold sub-area of the apparatus. The flexible nature of OTTER allows adapting the task for different species and research contexts.

OTTER offers a high temporal precision of the recall event making it a very promising task for a detailed study of retrieval mechanisms. There is a very small time-window when an animal retrieves information and acts upon it. Such pinpointing of the recall event is difficult in tasks where behavioral response is registered as a frequency of behavior during a time interval (freezing or exploration duration). Temporal precision of the recall event offered by OTTER can be especially advantageous if combined with methods using high temporal resolution, such as electrophysiology[19] or calcium imaging[20]. OTTER might therefore serve as a valuable behavioral paradigm for a detailed study of neural mechanisms involved in episodic-like memory retrieval.

We consider OTTER highly relevant to episodic memory because the successful recall of CS-2s-US in OTTER meets several criteria of episodic memory: a) the memory was incidentally encoded(6), b) encoding occurred after a single exposure[21], c) there was no pre-training involved [22], d) rat behavior observed threshold retrieval dynamics[23], and e) rats were able to retrieve information flexibly in a different context[24]. Considering these five criteria, OTTER is a good model of several putative aspects of episodic memory. However, OTTER does not meet the episodic-like memory criterion of demonstration of what-where-when knowledge of past experiences[25] because the flight in response to the CS does not indicate if the rat remembers where and when it experienced CS-2s-US. Rather than puzzling over whether OTTER is 'an episodic-like memory task' or not, we find it more helpful to focus on the fact that OTTER captures several important aspects of episodic memory and enables us to study them.

OTTER might also be utilized for the study of extinction of incidentally acquired memory based on a single exposure. This aspect is highly relevant for several neuropsychiatric disorders and seems

especially promising as an ecologically valid model of memory acquisition/extinction in post-traumatic stress disorder (PTSD). We expect that the OTTER extinction curve could be manipulated in both directions (faster/slower) by behavioral manipulations during or after the recall session.

As in any behavioral task, there are limitations to OTTER. First, the one-trial nature of OTTER precludes repeated measurements that are often required to accumulate sufficient amounts of data (as in electrophysiology). This limitation stems from probing the incidental one-trial aspects of episodic-like memory and seems to be unavoidable. Second, it cannot be ruled out that 'non-responders' did in fact form the CS-2s-US association but failed to act upon it. In assessing recall, we rely on motoric output that is only indirectly related to the mental state of an animal.

In conclusion, we designed a temporal binding task called OTTER that is adaptable, gives rapid results and is easy to conduct. Due to the association of temporarily discontinuous events, OTTER can be of great help in understanding the neural mechanisms of temporal binding and possibly memory extinction. The behavioral response in OTTER is ecologically valid because it takes advantage of the natural behavioral tendencies of rodents. We demonstrated that rats can utilize the knowledge acquired from a single past experience and use it to their advantage in a different context: rats demonstrated the same behavior that resulted in the termination of the unpleasant stimulus they experienced 24-hours earlier. OTTER extends the current range of trace conditioning tasks, capturing the one-trial and incidental nature of encoding, and offers high temporal precision as to when the memory recall occurred. Moreover, the OTTER task shares aspects with episodic memory due to its incidental, single-trial character with minimal training requirements. Another notable advantage of OTTER is its unambiguous binary outcome. The rat either crosses to the light sub-area or it does not, thus there is no need to set an arbitrary threshold for the outcome variable, as is needed for continuous outcome measures.

Methods

Altogether, 20 adult Wistar male rats were used for the OTTER experiment. Upon arrival at Anlab, the 28-day old rats were housed in standard laboratory cages (50 x 25 x 25 cm), two animals per cage. Laboratory food and tap water were supplied ad libitum. The room where the animals were kept was ventilated with a constant temperature of 22 °C and 50% humidity. The rats were kept on a 12-hour light cycle and lights were turned on daily at 6 am.

Rats were randomly assigned into two groups, the first group (N = 12) received handling by the experimenter daily for 2 minutes for 4 days preceding the start of OTTER, while the second group (N = 8) received no handling. All experiments were conducted in the light phase of the day because rats show lower locomotion during that time[26]. All animal procedures were approved by Ethical Committee of the Czech Academy of Sciences and complied with the Animal Protection Act of the Czech Republic and EU directive 2010/63/EC.

Apparatus

Two modified TSE multi conditioning shuttle boxes (TSE Systems GmbH, Germany) were used in the experiment. Each shuttle box consisted of two interconnected 24 x 47 cm sub-areas. The first sub-area of both shuttle boxes was built from transparent acrylic glass (light sub-area), while the second sub-area was created using dark opaque acrylic glass (dark sub-area). A dark opaque lid was used to cover the dark sub-area, which resulted in a light intensity of less than 3 lx in the center of the sub-area. The light sub-area was left uncovered; moreover, we added an additional light source to reach a light intensity of 1090 lx in the center of the sub-area. Intense light is highly uncomfortable for rodents[27] and aversively motivated rats spend most of their time in the dark sub-area. The sub-areas were separated by a custom made dark acrylic glass partition with a 4 x 40 cm wide central opening.

The shuttle boxes were soundproofed and equipped with a speaker. Once triggered by the TSE software, the speaker delivered a 2400 Hz sound cue. The sound cue was delivered at 80 dB SPL intensity for 2 seconds. The walls of both sub-areas were equipped with infrared devices which registered the location of the animal within the apparatus. The floor of both sub-areas consisted of a metallic grid with 0.5 cm diameter metal rods spaced 1.5 cm apart. When prompted, the metal rods delivered a 1.0 mA pulsatile electric stimulus with a 400 ms period (a 200 ms, 1 mA stimulus followed by 200 ms no stimulus), to the animal in the dark sub-area.

The two TSE multi conditioning shuttle boxes were visually and olfactorily distinct so that one shuttle box served as environmental context A and the other as environmental context B. In context A, the walls of the light sub-area were decorated with an aquarium scene on a circular insert, while in context B the walls were decorated with black stripes. The surfaces of the sub-areas were washed with an alcohol-based wash in context A, while a vinegar-based wash was used for cleaning in context B.

We presume that the OTTER task can be conducted using any similar apparatus where the above-described general principles are adhered to will deliver comparable results to those using TSE shuttle boxes.

Habituation, Pairing and Recall

Rats were individually habituated to each environmental context in two 15-minute daily sessions and the sessions in contexts A and B were alternated daily. At the start of the habituation, rats were first placed in the dark sub-area, then they were left to freely explore both sub-areas.

Following habituation, rats were conditioned to CS-2s-US pairing. The pairing took place in the same context as the first habituation session: rats that were exposed to context A first experienced CS-2s-US pairing in context A and vice versa for context B. The beginning of the pairing session closely resembled the habituation session where the rats were allowed to move freely through the apparatus for 15 minutes. At this point, the rats did not transfer between the sub-areas at all or only very infrequently. Following the 15-minute interval a to-be-conditioned stimulus, a sound cue, was delivered. The CS must be delivered cautiously as delivering the CS at an inappropriate moment might hamper the CS-2s-US acquisition. At the time of the CS the animal must be located in the dark sub-area and it should be resting and not facing

the door (to avoid bias toward escaping through the door). Two seconds after the termination of the CS, an electric foot-shock was delivered (US) from the metallic floor grid. The US was automatically terminated when the position of the rat was registered in the light sub-area or if the rat did not leave the dark sub-area in 20 seconds. Rats that did not escape were excluded and did not proceed to the recall session. Rats that did escape were retrieved from the light sub-area and returned to their home cage immediately after the escape. Rats were left undisturbed in their home cages for the next 24-hours, after which they were tested for recall.

The recall of the CS-2s-US pairing took place in the alternate context, i.e., if the pairing took place in context A, the recall was tested in context B. Recall session resembled the pairing session with the exception that the US was not delivered. The CS was delivered no sooner than after 15 minutes and only if the rat rested in the dark sub-area. Following the delivery of the CS, the response of the rat was observed. Rats that escaped to the light sub-area within 10 seconds of the CS start were considered 'responders', while those that remained in the dark sub-area were considered 'non-responders.'

Data visualization

Data visualizations were created in R using the visualization library ggplot2[28]. Heatmaps were obtained using the two-dimensional kernel density estimation function, kde2d from the MASS library[29].

Declarations

Data availability

The datasets generated and/or analysed during the current study are available in the GitHub, <https://github.com/hejtmy/fgu-otter-paper>

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AUTHOR CONTRIBUTIONS

Conceptualization, H.B., D.R. and B.K.; Methodology, D.R., T.P. and D.K.; Investigation, D.R. and D.K.; Resources, A.S and J.S.; Writing – Original Draft, D.R. and D.K.; Writing – Review & Editing, H.B., D.K., B.K., T.P. and L.H.; Visualization, L.H. and H.B.; Supervision, H.B. and A.S.; Funding Acquisition, A.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

References

1. Crystal, J. D. Animal models of episodic memory. *Comparative Cognition & Behavior Reviews* **13**, 105–122 (2018).
2. Tulving, E. Episodic memory: From mind to brain. *Annual Review of Psychology* **53**, 1–25 (2002).
3. DuBrow, S. & Davachi, L. Temporal binding within and across events. *Neurobiology of Learning and Memory* **134**, 107–114 (2016).
4. Zhou, W. & Crystal, J. D. Validation of a rodent model of episodic memory. *Animal Cognition* **14**, 325–340 (2011).
5. Zhou, W., Hohmann, A. G. & Crystal, J. D. Rats answer an unexpected question after incidental encoding. *Current Biology* **22**, 1149–1153 (2012).
6. Zentall, T. R. Animals represent the past and the future. *Evolutionary Psychology* **11**, 573–590 (2013).
7. Rugg, M. D., Fletcher, P. C., Frith, C. D., Frackowiak, R. S. J. & Dolan, R. J. Brain regions supporting intentional and incidental memory: a PET study. *NeuroReport* **8**, 1283–1287 (1997).
8. Trivedi, M. A. *et al.* fMRI activation changes during successful episodic memory encoding and recognition in amnesic mild cognitive impairment relative to cognitively healthy older adults. *Dementia and Geriatric Cognitive Disorders* **26**, 123–137 (2008).
9. Kuhnert, M.-T. *et al.* Incidental and intentional learning of verbal episodic material differentially modifies functional brain networks. *PLoS ONE* **8**, e80273; 10.1371/journal.pone.0080273 (2013).
10. Wang, W.-C. & Giovanello, K. S. The role of medial temporal lobe regions in incidental and intentional retrieval of item and relational information in aging. *Hippocampus* **26**, 693–699 (2016).
11. Keller, F. S. Light-aversion in the white rat. *The Psychological Record* **4**, 235–250 (1941).
12. Fanselow, M. S. Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review* **1**, 429–438 (1994).
13. Wendt, J., Löw, A., Weymar, M., Lotze, M. & Hamm, A. O. Active avoidance and attentive freezing in the face of approaching threat. *NeuroImage* **158**, 196–204 (2017).
14. McEchron, M. D., Cheng, A. Y. & Gilmartin, M. R. Trace fear conditioning is reduced in the aging rat. *Neurobiology of Learning and Memory* **82**, 71–76 (2004).
15. Sharma, V. *et al.* Trace fear conditioning: procedure for assessing complex hippocampal function in mice. *Bio-protocol* **8**, e2475; 10.21769/BioProtoc.2475 (2018).
16. Blanchard, D. C., Griebel, G., Pobbe, R. & Blanchard, R. J. Risk assessment as an evolved threat detection and analysis process. *Neuroscience & Biobehavioral Reviews* **35**, 991–998 (2011).
17. Sato, N. Episodic-like memory of rats as retrospective retrieval of incidentally encoded locations and involvement of the retrosplenial cortex. *Scientific Reports* **11**, 2217; 10.1038/s41598-021-81943-9 (2021).
18. Park, C. R., Zoladz, P. R., Conrad, C. D., Fleshner, M. & Diamond, D. M. Acute predator stress impairs the consolidation and retrieval of hippocampus-dependent memory in male and female rats. *Learning & Memory* **15**, 271–280 (2008).

19. Kim, K. *et al.* Artifact-free and high-temporal-resolution in vivo opto-electrophysiology with microLED optoelectrodes. *Nature Communications* **11**, 2063; 10.1038/s41467-020-15769-w (2020).
20. Scott, B. B. *et al.* Imaging cortical dynamics in GCaMP transgenic rats with a head-mounted widefield microscope. *Neuron* **100**, 1045-1058.e5; 10.1016/j.neuron.2018.09.050 (2018).
21. Morris, R. G. Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **356**, 1453–1465 (2001).
22. Binder, S., Dere, E. & Zlomuzica, A. A critical appraisal of the what-where-when episodic-like memory test in rodents: Achievements, caveats and future directions. *Progress in Neurobiology* **130**, 71–85 (2015).
23. *Learning and Motivation* **36**, 190–207 (2005).
24. Clayton, N. S., Bussey, T. J. & Dickinson, A. Can animals recall the past and plan for the future? *Nature Reviews Neuroscience* **4**, 685–691 (2003).
25. Clayton, N. S., Bussey, T. J., Emery, N. J. & Dickinson, A. Prometheus to Proust: the case for behavioural criteria for ‘mental time travel.’ *Trends in Cognitive Sciences* **7**, 436–437 (2003).
26. Borbély, A. A. & Neuhaus, H. U. Daily pattern of sleep, motor activity and feeding in the rat: Effects of regular and gradually extended photoperiods. *Journal of Comparative Physiology* **124**, 1–14 (1978).
27. Barker, D. J. *et al.* Brief light as a practical aversive stimulus for the albino rat. *Behavioural Brain Research* **214**, 402–408 (2010).
28. Wickham, H. *ggplot2: Elegant graphics for data analysis* (Springer, New York, NY, 2016).
29. Venables, W. N. & Ripley, B. D. *Modern Applied Statistics with S*. (Springer, New York, NY, 2002).

Figures

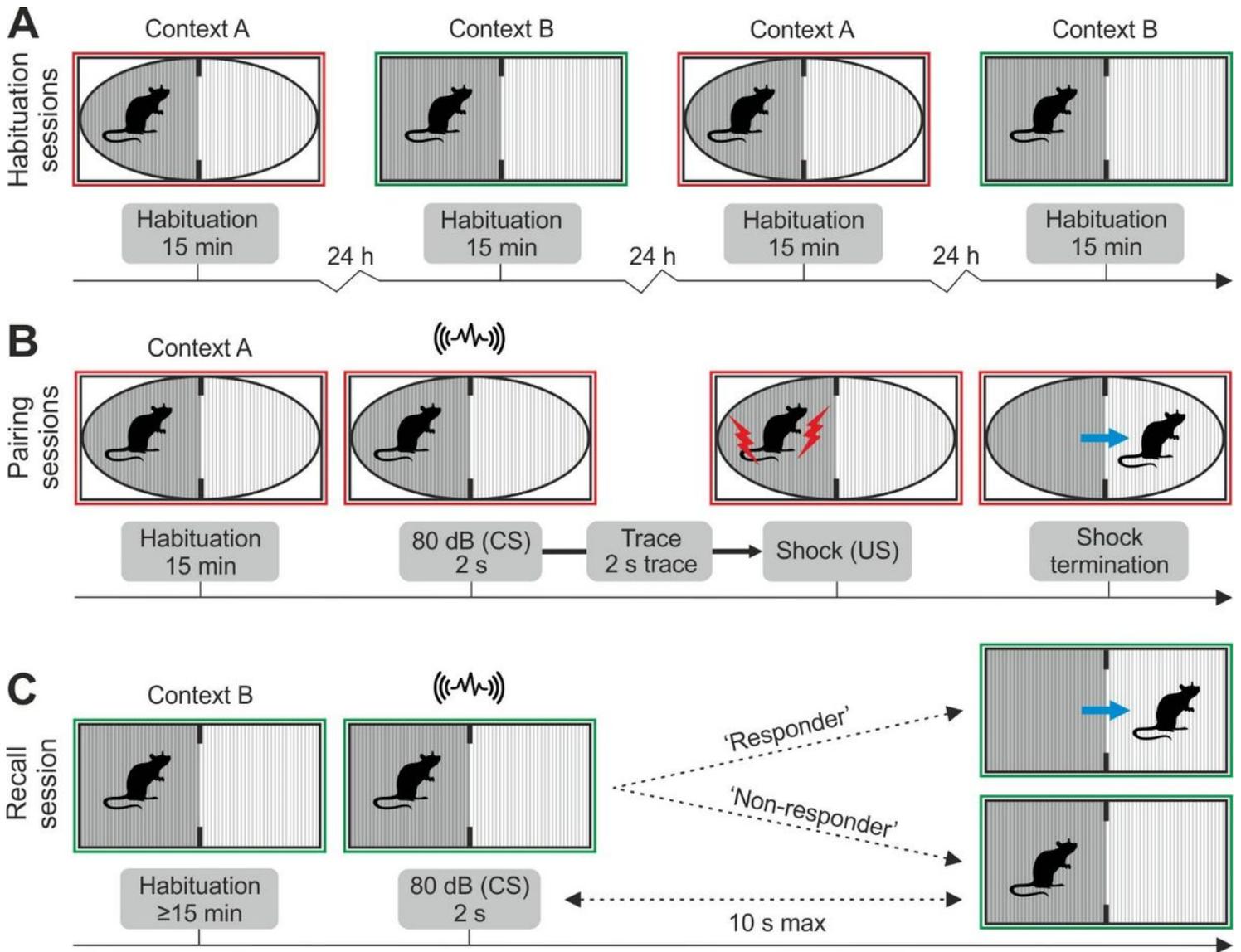


Figure 1

Schematic overview of OTTER. **(A)** A rat is initially habituated to environmental contexts A and B in a series of alternating daily sessions. **(B)** During the pairing session, the rat hears a sound cue (CS) while in the dark sub-area of one of the two contexts (context A or B); two seconds later the rat receives a foot shock (US) terminated once the rat transfers to the light sub-area. **(C)** After 24 hours, the association of CS with US is tested in the recall session occurring in the alternate context (context B or A). When the rat settles in the dark sub-area, the CS is delivered, and reaction observed. Rat either escapes into the light sub-area ('responder') or stays in the dark sub-area ('non-responder').

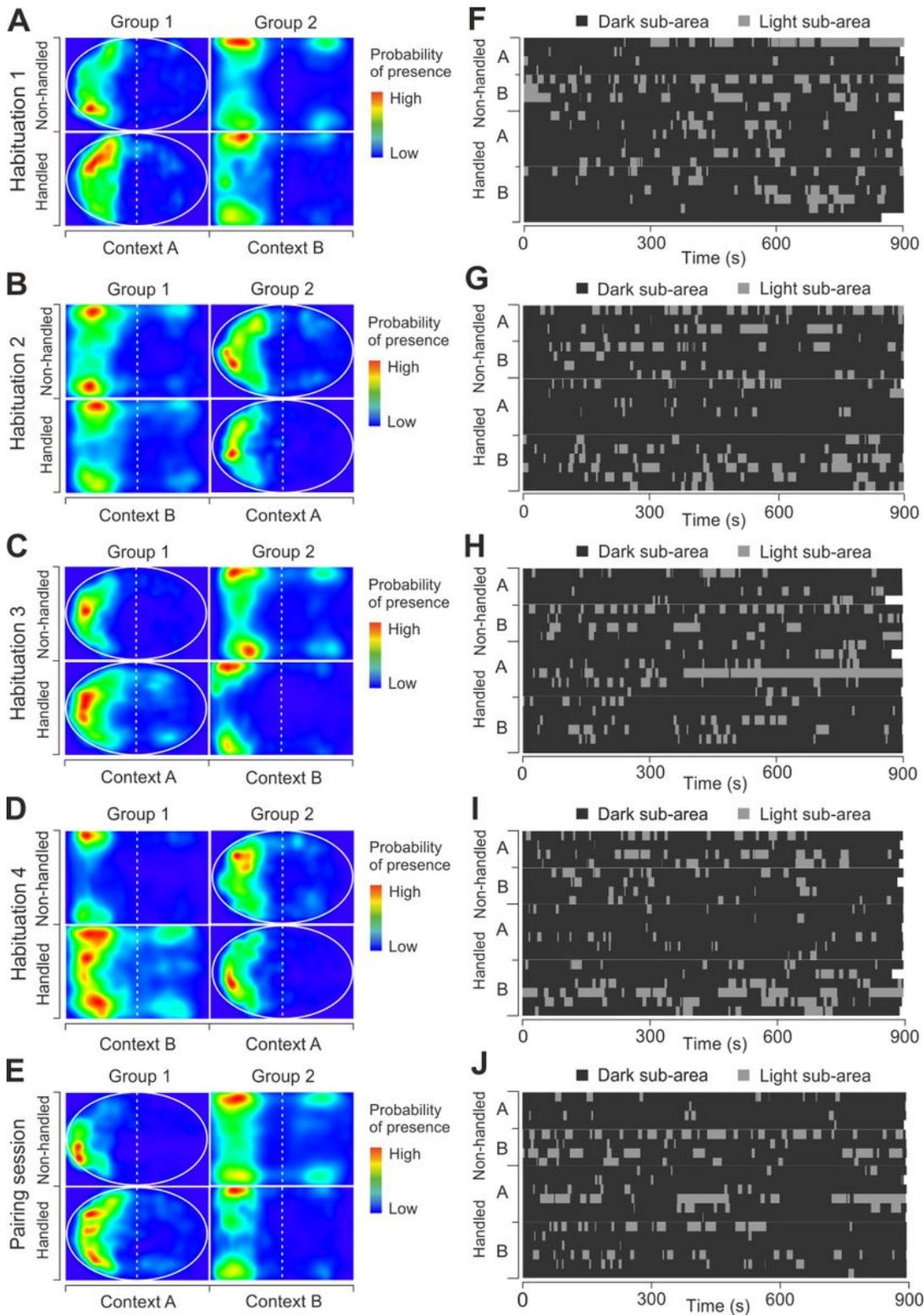


Figure 2

Sub-area preference and movement through the apparatus during habituation and pairing sessions. (A–E) Where animals were likely to be during each session, red signifies a frequent stay, a blue indicates minimal presence. Rats were most often present in the dark sub-area of context A and B during each session. (F–J) Location of rat (vertical axis) at a specific time during habituation sessions (seconds, horizontal axis). Black represents the dark sub-area; gray represents the light sub-area.

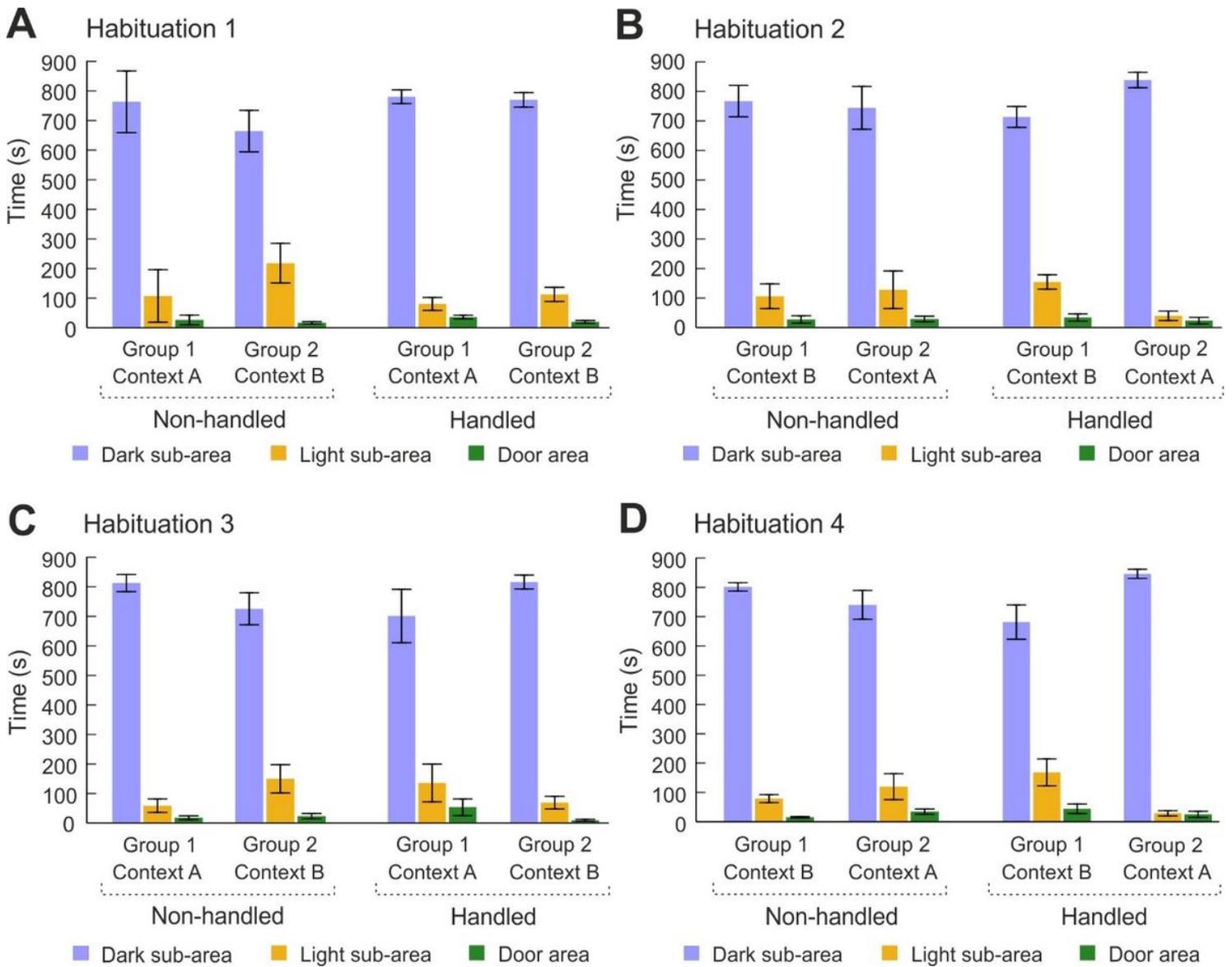


Figure 3

Time spent in sub-areas of contexts A and B by handled and non-handled rats during habituation sessions. (A–D) Each rat (N = 12 handled, N = 8 non-handled) received two habituation sessions per context in an alternating manner. Starting context was chosen randomly for each rat. Rats preferred the dark sub-area (blue bar) and spent very little time in the light sub-area (yellow bar) in both contexts across habituation sessions. Handling treatment did not affect sub-area preference. The green bar represents time spent between sub-areas ('door area'). Error bars indicate SEM.

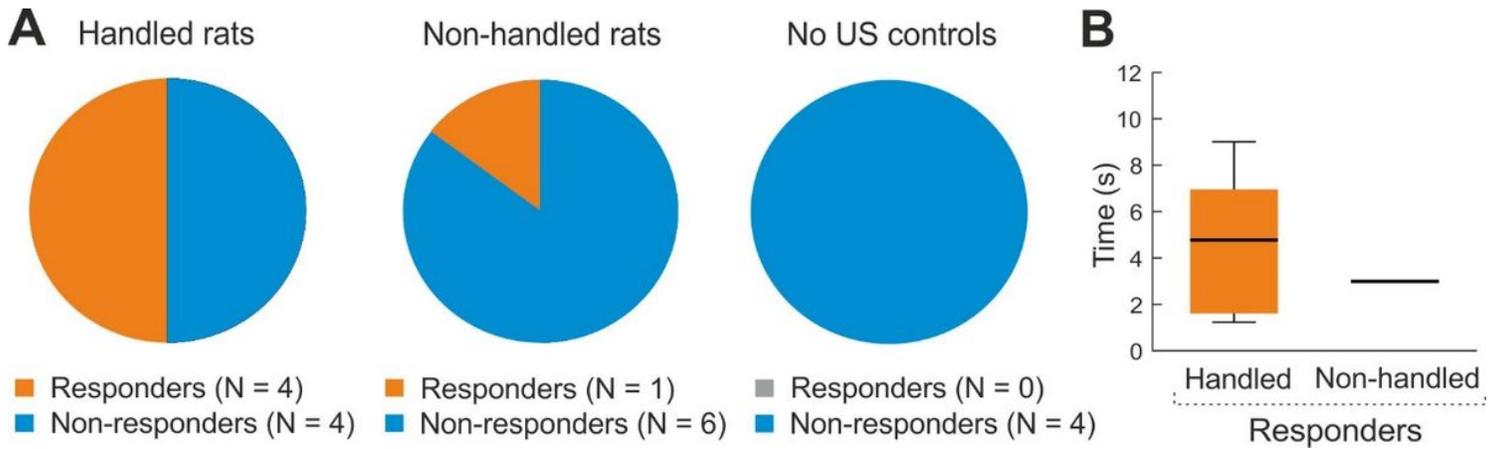


Figure 4

Effect of handling on performance in OTTER. **(A)** Ratios of responders (orange) to non-responders (blue) in handled (N = 8) and non-handled (N = 7) rats which received CS-2s-US pairing, and in rats that received CS only (controls, N = 5). Handling increased the ratio of responders to non-responders from 1:7 to 1:1. **(B)** Time to translocate to the light sub-area following the CS presentation during recall session. Average time to escape was: 4.9 ± 3.3 seconds in handled rats and three seconds in the non-handled rat. Error bars indicate SEM.

Supplementary Files

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