

Short communication

Transient inactivation of the anterior cingulate cortex in rats disrupts avoidance of a dynamic object



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ARTICLE INFO

Article history:

Received 7 August 2016

Revised 5 December 2016

Accepted 4 January 2017

Available online 6 January 2017

Keywords:

Enemy avoidance

Moving goal

Navigation

Avoidance

Rat

Robot

ABSTRACT

Although animals often learn and monitor the spatial properties of relevant moving objects such as conspecifics and predators to properly organize their own spatial behavior, the underlying brain substrate has received little attention and hence remains elusive. Because the anterior cingulate cortex (ACC) participates in conflict monitoring and effort-based decision making, and ACC neurons respond to objects in the environment, it may also play a role in the monitoring of moving cues and exerting the appropriate spatial response. We used a robot avoidance task in which a rat had to maintain at least a 25 cm distance from a small programmable robot to avoid a foot shock. In successive sessions, we trained ten Long Evans male rats to avoid a fast-moving robot (4 cm/s), a stationary robot, and a slow-moving robot (1 cm/s). In each condition, the ACC was transiently inactivated by bilateral injections of muscimol in the penultimate session and a control saline injection was given in the last session. Compared to the corresponding saline session, ACC-inactivated rats received more shocks when tested in the fast-moving condition, but not in the stationary or slow robot conditions. Furthermore, ACC-inactivated rats less frequently responded to an approaching robot with appropriate escape responses although their response to shock stimuli remained preserved. Since we observed no effect on slow or stationary robot avoidance, we conclude that the ACC may exert cognitive efforts for monitoring dynamic updating of the position of an object, a role complementary to the dorsal hippocampus.

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1. Introduction

Tremendous progress in understanding neural mechanisms and the substrates of spatial learning and memory has been fueled by cognitive map theory (Nadel & O'Keefe, 1978; Tolman, 1948). However, albeit complex and comprehensive, this framework did not cover one important aspect of animal navigation that had remained neglected until recently – spatial behavior in relation to moving (dynamic) cues. It is now well recognized that introducing a continuously moving element into an animal's environment adds another level of complexity. Rats apparently organize their spatial behavior in relation to conspecifics (Dorfman, Nielbo, & Eilam, 2016), predators (Blanchard, Griebel, Rodgers, & Blanchard, 1998), or moving artificial objects (Shi et al., 2013), and it has been demonstrated that prey-predator distance determines the response elicited (Blanchard et al., 1998). Therefore, rats

easily remember the particular distance to a dynamic cue under laboratory conditions (Ho et al., 2008; Wilson et al., 2015).

Efforts to find a distinct neural correlate have yielded only partial success. Firing of hippocampal pyramidal cells does not exhibit clear correlation with the position of a moving toy car (Ho et al., 2008), or even a naturally significant object such as a conspecific (von Heimendahl, Rao, & Brecht, 2012; Zynjuk, Huxter, Muller, & Fox, 2012). However, place cells do respond to independently moving parts of an environment (Kelemen & Fenton, 2013, 2016). Quite recently, it was seen that a conspecific exerts a much more powerful effect on place cells when CA2 was targeted (Alexander et al., 2016). Furthermore, a predator-like threatening object significantly modulates place fields in its vicinity (Kim et al., 2015). Previously, we developed two aversively motivated behavioral tasks allowing the investigation of spatial learning and memory in relation to a moving object (Telensky et al., 2011, 2009). Our principal finding was that the dorsal hippocampus plays a critical role in maintaining a safe distance from a moving robot but not a immobile robot (Telensky et al., 2011). So far it is unclear whether other brain structures participate in organizing spatial behavior in relation to a moving object and whether the network expands beyond

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the hippocampus. One of the candidate structures would be the anterior cingulate cortex (ACC) for the following reasons. Ideally located to combine emotional, visceral, and highly processed cognitive input, the ACC has been shown to be implicated in error detection (Carter et al., 1998), cognitive control (Carter & van Veen, 2007), and effort-based decisions (Hauber & Sommer, 2009). Neurons of the ACC respond to the presence of objects in an environment (Weible, Rowland, Monaghan, Wolfgang, & Kentros, 2012; Weible, Rowland, Pang, & Kentros, 2009). Maintaining a safe distance from a moving object would require dynamic monitoring of the perceived distance, threat level, and the spatial context that would allow the organization of fast escape responses. As it was shown in gerbils, the process of selecting an appropriate escape trajectory might be multi-leveled. Gerbils can compute the optimal trajectory leading to a refuge by taking into account the position of the threat, the location of a clearly visible refuge and several other contextual variables (Ellard & Eller, 2009). Human studies suggest the involvement of the ACC in evaluations of the spatial imminence of a threat stimulus (Mobbs et al., 2009). The objective of this study was therefore to determine the involvement of the ACC in avoidance of a moving object in laboratory rats. We used a robot avoidance procedure (Svoboda, Telensky, Blahna, Bures, & Stuchlik, 2012; Telensky et al., 2011). Since we believe active robot avoidance includes processes such as cognitive control and effort based decisions, we predicted that an inactivated ACC would interfere with avoidance of a moving robot (particularly at high speeds) but not a stationary robot.

Ten male Long Evans rats (Charles River, Italy) were 3 months old upon arrival and were given at least 14 days for acclimatization prior to surgery. They were housed in groups of two or three in plastic cages and provided with food and water *ad libitum*. To implant cannulae over the ACC, rats were placed under isoflurane anesthesia, had heads shaved, placed in a stereotaxic frame, and the scalps were retracted. Two holes were drilled at +2.0 AP and ± 0.7 ML (relative to *bregma*) through which custom-made stainless steel cannulae (22 gauges, 11 mm; with stylets) were inserted so that their tip remained 2 mm below the skull surface. Two anchoring screws were mounted frontally. Both screws and cannulae were embedded with dental cement, and the wound was sutured. Rats were given intensive postoperative care (antibiotics, analgesics) and left at least 14 days for recovery. Behavioral training consisted of 20-min daily sessions taking place in an elevated circular metallic arena ($d = 130$ cm), enclosed with an opaque wall. The procedure is similar to that described in our previous studies (Svoboda et al., 2012; Telensky et al., 2011). Briefly, once a rat was placed in the arena, it

was required to maintain a safe distance of at least 25 cm from the center of a custom-made programmable robot (Fig. 1A, B).

Custom-made software (Kachna tracker) tracked the position of both the rat (wearing a small LED) and the robot (with a larger LED), and delivered a mild foot-shock (with adjustable intensity 0.2–0.8 mA) whenever the robot-rat distance dropped below 25 cm. The robot was programmed to move straight until it hit the wall. Then it waited for 15 s, moved backwards 10 cm and turned at a random angle between 100 and 200 degrees. The robot speed was set to either 4 cm/s (a fast-moving robot), 0 cm/s (a stationary robot), and 1 cm/s (a slow robot). Rats were exposed to the fast-moving robot condition for 7 sessions, the stationary robot for 5 sessions, and the slow robot for 5 sessions (Fig. 1D). Each session lasted 20 min. In each condition, after the initial sessions, there then followed an inactivation session on the penultimate day and a saline session on the last day. Twenty minutes prior to the beginning of the inactivation session, rats were given muscimol (1 $\mu\text{g}/\mu\text{l}$ in saline; Sigma Aldrich). Each rat was gently restrained by hand while muscimol (volume of 0.5 μl) was infused (at a rate 0.5 μl per minute, with 1 min of a rest) manually into the ACC via an injection cannula (30-gauge stainless steel cannulae, 12 mm long) that was lowered through the implanted guide cannula. The injection cannula protruded 1 mm below the tip of the guide cannula. Once animals completed all sessions, they were deeply anesthetized with ketamine and xylazine, infused with 0.5 μl of black ink into the ACC through the guide cannulae, and perfused transcardially with 0.1 M PBS, pH 7.4, followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. The brains were post-fixed in PFA overnight, left cryoprotected in 30% sucrose until they sunk, and stored at -80°C for further processing. Free floating sections (50 μm) were cut using a Leica cryostat and Nissl stained using a standard protocol. The location of ink infusions was verified under a light microscope. For each experimental stage data were evaluated separately, using one-way ANOVA with days as repeated measures followed by Sidak's multiple comparisons *post-hoc* test when appropriate. A mixed two-way ANOVA was conducted on the rat-robot distance distribution with distance bins as repeated measures and a session (muscimol, saline) as main factor. When the data violated the assumptions of homoscedasticity, they were transformed using the natural logarithm. For comparing muscimol and saline sessions only, a paired *t*-test was conducted. GraphPad Prism (GraphPad software, Inc.) was used to perform statistics and the level of significance was set at $p < 0.05$.

During behavioral training to avoid the fast-moving robot, all rats gradually acquired efficient avoidance behavior and

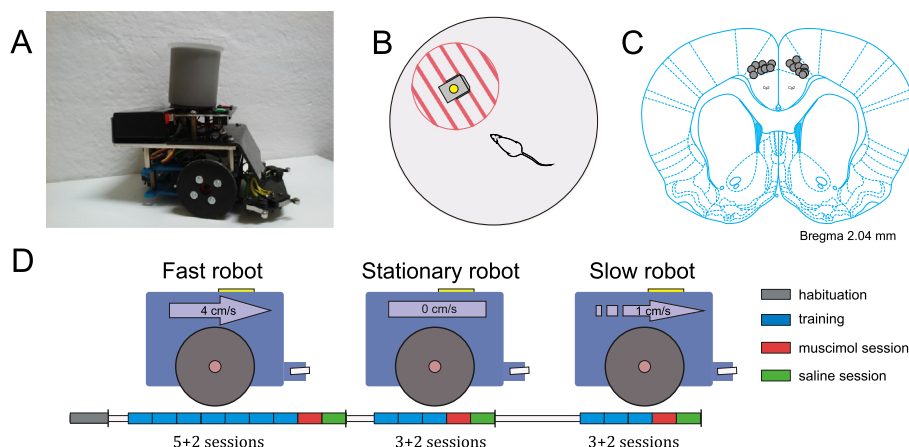


Fig. 1. Behavioral task. (A) Picture of the robot, (B) scheme of the apparatus with the shock zone ($d = 25$ cm) depicted, (C) placement of the tips of the injecting cannula (gray circles), and (D) time-scheme of the experiment (D). Coronal section diagram with cannula tip locations is based on the atlas of Paxinos and Watson (2006).

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