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Object Recognition - The Role of Hormones Throughout the Lifespan

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1. Introduction

There are several tasks that are used in behavioral neuroscience to reveal the neurobiological underpinnings of learning and memory processes. A task which has been gaining even more widespread use in recent years is the spontaneous object recognition task. The spontaneous object recognition task (heretofore referred to as the object recognition task) was developed for rats over 20 years ago, and has since been modified for use in mice (Dodart et al., 1997; Ennaceur and Delacour, 1988; Messier, 1997; Steckler et al., 1999). The background on this task, typical methods and methodological issues, and representative data obtained, when using this task to assess learning and memory processes in rodent models, will be reviewed in the following sections.

The object recognition task is considered a non-spatial working, declarative memory task. Performance in this task relies upon a functioning cortex and hippocampus. For a thorough review of the brain regions and neurotransmitters involved in object recognition task performance, readers are referred to recent papers on this topic (Dere et al., 2007; Winters et al., 2008). Unlike other tasks that typically rely on aversive stimuli or food rewards, the object recognition task takes advantage of the natural affinity of rodents for novelty (and see review on other methodological and theoretical considerations by Ennaceur, 2010). Although the typical stimuli used in this task are objects of different shapes and complexity, our laboratory has also begun to assess rodents' behavior using more socially-relevant stimuli, such as cagemates and novel conspecifics. In this review, data are presented demonstrating typical patterns of investigation when objects of different complexity, or conspecifics, are utilized as target stimuli in this task. The objective of this report is to review the utility of this task to assess socially- and non-socially relevant stimuli to reveal neurobiological underpinnings (e.g. hormones being of the greatest interest for us) for cognitive processes across the lifespan. The typical methods used in training and testing and assessing performance in this task will be reviewed and are as follows.

2. Training trial

Training in the object recognition task typically involves one training trial. In the case of object recognition memory, as a measure of declarative memory, acquisition is thought to

occur with less exposure to the stimuli to be learned/recognized than in the case of non-declarative memory (e.g. procedural memory for a skill). Training in the object recognition task involves exposing rodents to two stimuli. In a typical training trial in this task, rats or mice are trained in a bright open field (for rats: 45 × 24 × 21 cm; for mice: 39 × 39 × 30 cm) with two identical objects as the target stimuli in each of the corners of task that are furthest from where the rodent is introduced to the chamber. Another approach that our laboratory has been using to investigate socially-relevant cognitive processes is to use conspecifics as the training stimuli in this task. Rodents readily explore these novel objects, or other rodent conspecifics, during the training session and the amount of time spent exploring the objects is recorded.

Objects are readily approached and then explored (touching, manipulating, sniffing, climbing/rearing upon) by rodents (Aggelton, 1985). Exploration is operationally defined as the rodent directing its nose at the object at a distance of no greater than 1 cm and/or touching, or climbing on, the object. Rodents typically spend equal amounts of time exploring both objects, or conspecifics, during training. It is important to take into account any preference for one object over another in the training trial. Further discussion of the importance of assessing preferences for objects utilized in the object recognition task is in Section 6 below.

The length of the training trial that we have used with consistent results to be able to assess cognitive performance and mnemonic effects of hormones of rats and mice is three-minutes. Other laboratories have utilized 2-10 minutes for the training trial (reviewed in Dere et al., 2007). Another variation in training trials is that the length of the training trial is based upon animals reaching a pre-set criterion for duration spent investigating the objects (e.g. 30 seconds total exploration time; Frick & Gresack, 2003). A typical inclusion criterion is that subjects spend time exploring each stimuli during training. Valid interpretations cannot be made if rodents do not explore both objects sufficiently during training.

3. Retention Interval

As with the training trial length, the retention interval is an important consideration to make when using the object recognition task. Although the typical retention intervals that are utilized are between 3 and 24 hours, some studies have used retention intervals spanning days (Dere et al., 2007). Rodents' performance in the task is better with shorter retention intervals (Bertaina-Anglade et al., 2006; Dere et al., 2007; Obinu et al., 2002; Schiapparelli et al., 2006), but with intervals shorter than 3 hours, it has been argued that it is not possible to make any attributions about rodents' cognitive performance beyond that they are able to perform the task and investigate the objects (Baker and Kim, 2002; Winters and Bussey, 2005b). Furthermore, forgetting in this task is dependent not only on the retention interval, but other factors, such as the length of the training trials and rodent species and strain used. In our laboratory, we utilize a 4 hour retention interval. This is done because in studies of natural cyclical variations in ovarian or other steroids (glucocorticoids, etc), it can be important to train rodents in the same hormone state as they will be tested in. For example, with respect to female rodents, the estrous cycle phase is 4 days long. In other studies using different learning tasks, we found that it was important to have a short enough retention trial so that they are trained in the same hormone state as when they are tested in (Frye, 1995; Rhodes and Frye, 2004). Indeed, the object recognition task assesses memory for a unique episode or event, and has been argued to be more sensitive to pharmacological or

other manipulations that are amnestic (Dere et al., 2007). However, the nature of training and retention trials can be modified so that the effects of amnestic as well as memory-enhancing effects of manipulations can be determined (Ennaceur & Meliani, 1992a; Ennaceur et al., 1989). As such, we have found valid and reliable results utilizing a three-minute training trial with a four-hour retention interval in the object recognition task.

4. Testing trial

Testing in the object recognition task involves assessing whether rats or mice spend more time exploring the novel stimuli, compared to the familiar stimuli they were exposed to during training. After a retention interval, subjects are placed in the same open field, which contains one of the stimuli encountered during training and one novel stimuli. The side of the open field that the novel object, or conspecific, is placed is counterbalanced across subjects in the event of a side bias of the subjects. The testing session is typically the same length as the training session, which is three-minutes in our laboratory. During the testing session, the duration of time rats or mice spend exploring the familiar and novel stimuli are recorded.

An assessment of performance in this task is done by comparing the amount of time exploring the novel object versus the familiar stimuli. This is often calculated as a percentage of total time spent exploring to take into account differences between subjects in exploration of the stimuli during testing. Chance levels of performance in this task are 50% of time spent exploring the novel stimuli during testing. Improved performance in this task is supported by greater than 50% time spent exploring the novel stimuli in this task.

5. Subjects

The object recognition task was developed in rats and can be used with mice with only modest modifications (Dodart et al., 1997; Ennaceur and Delacour, 1988; Messier, 1997; Steckler et al., 1999). As with other learning tasks (Frick et al., 2000; Whishaw and Tomie, 1996), there are differences between mice and rats in the object recognition task. Few studies have directly compared performance of rats and mice in the object recognition task. In one, both male Sprague-Dawley rats and C57Bl/6J mice were sensitive to the amnestic effects of scopolamine, but there were differences in the length of the retention trial in which this became apparent (Bertaina-Anglade et al., 2006). Generally, mice spend less time exploring the objects and approach the objects less (Dere et al., 2007). It is argued that this may be due to greater neophobia of objects among mice (Dere et al., 2004). One way to increase exploration of the objects in this task is to introduce a habituation phase so that rodents have been exposed to the open field prior to testing. This may reduce neophobia as well as reduce the time rodents spend exploring the testing chamber, rather than the stimuli. Thus, rats and mice can be used in object recognition, but there are species characteristics to consider when using this task and interpreting the results gained from it.

Another characteristic of subjects to consider is the strain of rodents utilized. For example, we have primarily utilized Long-Evans rat and mice on a C57Bl/6 background in our laboratory. These strains are pigmented and, thus, likely have greater visual abilities in this task, which may increase time spent exploring and/or improve recognition in the object recognition task. Few studies have systematically investigated strain differences among mice. In one, BALB/C, C3H/He, DBA/2, C57BL/6J, CBA/Ca, and 129S2/Sv mice were

compared (Brooks et al., 2005). Mice were able to perform this task with a 1 and 4, but not 24 hour, retention interval, with the BALB/c and DBA/2 strains spending a greater percentage of time exploring the novel object during testing. Of note, there were no differences between strains when the absolute amount of time exploring the novel object was compared. Other studies have noted that C57Bl/6 mice perform better than DBA/2 mice in object recognition (Podhorna & Brown, 2002; Voikar et al., 2005). Thus, strain of mice and rats must be considered with respect to experimental design and interpretation of results using the object recognition task.

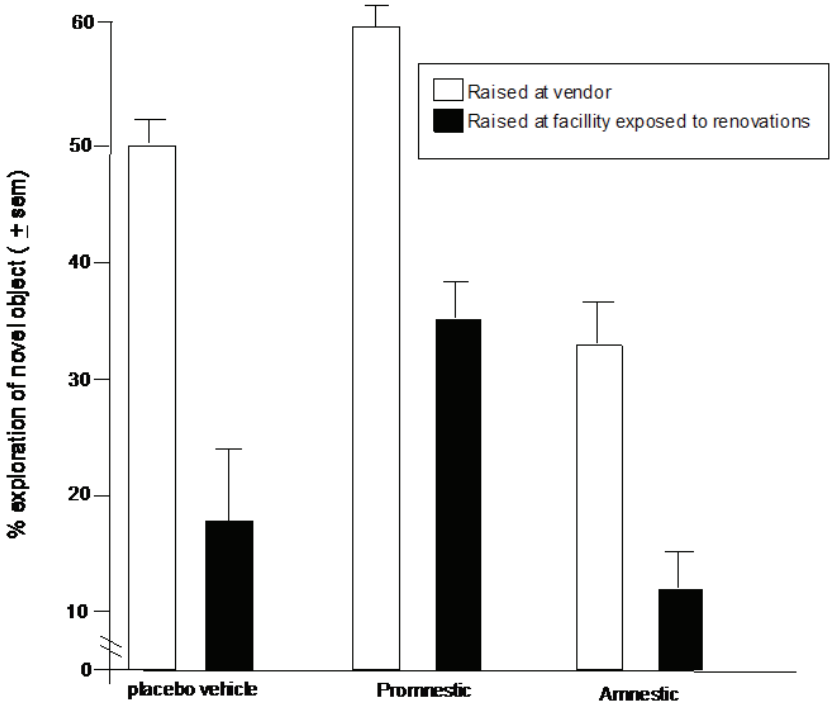

Fig. 1. Behavioral data in the object recognition task of ovariectomized female mice from two sources- raised at a vendor, or purchased from vendor and raised in brand-new facility with noise from renovations. Mice were administered placebo vehicle or a promnestic (estradiol) or an amnestic (scopolamine).

Another question to consider is the source and experiential effects of subjects. We have recently found differences among substrains of C57Bl/6 mice in that those that were raised by a vendor (C57Bl/6Tac) outperformed those that had been purchased from a vendor and raised in our facility (C57Bl/6J) that had renovations ongoing (e.g. frequent fire alarms unintentionally sounding, drilling, etc.), that can be typical of brand-new buildings (Figure 1). As well, the magnitude of effects when mice were injected systemically with a promnestic (estradiol) or an amnestic (scopolamine) was different between these substrains of mice. We are currently investigating these effects and the role of hormones further. Thus, sources and experiential effects must be considered for object recognition.

6. Non-socially-relevant stimuli- objects

A critical aspect of the object recognition task is the stimuli that are utilized (i.e. objects). Rodents must have some preference for the objects used and readily investigate them during training and testing trials. They need to be washable to remove extraneous olfactory stimuli. Likewise, the same type of material should be used (plastic, metal, etc). Similar size objects that differ on shape, color, texture, and/or height are preferable so that objects are different enough so that they can be discriminated. However, it is important that during training and testing objects of similar valence are used so that results are not confounded by a clear preference for one object over another (irrespective of recognizing the novelty or familiarity of the object). In our laboratory, we have analyzed the preference of rats and mice for several objects so that objects are ones those subjects readily investigate for equal amounts of time. A description of these data in mice is as follows.

The objects that we use in our laboratory are made of plastic and are similar size, but have different shapes, colors, and textures. We investigated the average amount of time (seconds) that mice spent exploring objects for three minutes in the open field box. Table 1 depicts the objects analyzed and the mean time spent by groups of mice exploring the objects. These data show that the amount of time mice spend exploring these objects varies across the types of objects assessed. In this example, it would not be preferable to use either the objects that the mice spent a the shortest or longest duration exploring, but rather those objects that mice explored similarly explored for a moderate time so that comparisons between novel and familiar objects could be assessed. By systematically investigating the amount of

Objects	Average Time Explored (seconds)
Apples (toy)	1.9
Blocks	5.0
Buoys (toy)	13.2
Cakes (toy)	7.8
Caps	24.2
Chilies (toy)	6.0
Funnels	8.1
Hydrants (toy)	16.2
Ketchup bottles (toy)	6.7
Lego- large (toy)	0.1
Lego- medium (toy)	0.5
Lego- small (toy)	0.9
Mice	10.2
Oranges	5.5
Pears (toy)	5.3
Pipes	39.1
Soda bottles (toy)	1.4
Water Bottles (toy)	24.8

Table 1. Time spent investigating objects of different complexity by mice.

exploration for all objects to be used for object recognition, it can be determined whether or not objects are ideal to use in an experiment. The ideal objects for use elicit a reliable exploratory response from the mice that can be differentiated from each other. It is advisable to have a catalogue of validated objects for rats and mice. If more objects are needed, they should approximate the characteristics of these existing objects, and be validated. Thus, when setting up the object recognition task to assess cognitive performance of rodents, it is essential to validate and catalogue a number of different objects to utilize.

7. Cognitive performance across the lifespan- role of hormones

Object recognition performance using the methods described above is influenced by hormones. There is evidence for sex differences, and effects of hormone extirpation/removal and replacement for object recognition performance, which suggests that hormones influence performance in this task. There are sex differences in that females typically outperform males in object recognition performance, but males outperform females when the objects are moved to different locations in the testing chamber (spatial version of object recognition referred to as the object placement task; Bowman et al., 2003; Ceccarelli et al., 2001; Sutcliffe et al., 2007). A question that has been of interest in our laboratory is the extent to which some of these effects may be related to effects of ovarian steroids. When rats or mice are tested during the estrous cycle, performance is best when there are natural elevations in estradiol and progestogens (progesterone and its neuroactive metabolites), as compared to their counterparts with low levels of these steroids (Walf et al., 2006; Walf et al., 2009). Ovariectomy (which removes the main peripheral source of estradiol and progestogens) impairs object recognition performance, and this is reversed with replacement back with physiological levels of estradiol or progestogens immediately after training (Walf et al., 2006). Interestingly, these steroids need to be "on-board" during the consolidation phase of memory formation, which occurs within the 1 or 1.5 hours post-training. If steroid administration is delayed to 1-1.5 hours post-training, then performance is not enhanced when rodents are tested 4 hours after training (Frye & Walf, 2008a). Thus, these data support a role of ovarian steroids for object recognition performance.

Performance of rats administered different pharmacological treatments, or mice that are genetic knockouts for steroid targets of interest, in the object recognition task has been used to investigate the mechanisms of steroids for learning and memory in the object recognition task. Data suggest that the traditional target of progesterone, the intracellular progestin receptor, is not required for progestogens' mnemonic effects, but metabolism may be (Frye & Walf, 2010; Frye et al., 2010). Similarly, there may be non-traditional targets of estrogens for object recognition performance. Although selective estrogen receptor modulators that act at estrogen receptor β and the traditional target of estrogens, estrogen receptor α, can improve performance in this task, estrogens do not improve performance of mice that have had estrogen receptor β knocked out (Jacome et al., 2010; Luine et al., 2003; Walf et al. 2008; 2009). Thus, there may be non-traditional actions of steroids for object recognition performance.

The subjects of these studies, discussed above, were young rodents. A question is the extent to which there are age-related changes in performance in object recognition that occur concomitant with decline in ovarian steroids. First, of interest is whether prior hormonally-relevant experiences may alter later effects of hormones for cognitive performance. To investigate this, age-matched rats with different breeding histories (no, one, or multiple past

pregnancies) are compared. We, and others, have demonstrated that middle-aged rats that have experienced past pregnancies have improved performance in the object recognition task compared to those that have not experienced such breeding history (Macbeth et al. 2008; Paris & Frye, 2008). Second, of interest is whether older subjects, with reductions in natural variations in steroids, can respond to hormone replacement. We have found that middle-aged rats with declining reproductive status, and lowered capacity to metabolize natural steroids, have worse performance than age-matched rats that have maintained reproductive status (Paris et al. 2010). Further, administration of the hormone therapy, conjugated equine estrogens, to middle-aged rats improves performance in the object recognition task (Walf & Frye, 2008). Among aged mice, administration of progesterone acutely after training improves object recognition performance (Frye & Walf, 2008b). As well, long-term administration of progesterone to transgenic mice with an Alzheimer's Disease-phenotype, or their normative age-matched controls, improved performance in the object recognition task (Frye & Walf, 2008c). Together, these data demonstrate that there is a role of hormones across the lifespan for object recognition performance.

8. Socially-relevant stimuli- conspecifics

Given the clear role of ovarian steroids for object recognition performance, described above, as well as their well-known actions to mediate socially-relevant behaviors (reviewed in Frye, 2009), of interest is designing a one-trial learning task to assess memory for socially-relevant stimuli, such as conspecifics. We have recently been using a modified version of the object recognition task, where, instead of objects as stimuli, novel and familiar conspecifics are utilized. All other aspects of the protocol are the same in terms of the testing chamber utilized, and lengths of the training trial, retention interval, and testing trial. Rodents are trained with two of their cagemates in each corner of the open field. The cagemates are placed under separate screened chambers. The experimental subject can then see and smell, but not touch, the conspecifics. The operational definition of exploring in this case is defined as the rodent touching or directing its nose at the chamber containing the conspecific at a distance of no greater than 1 cm. Rodents typically spend equal amounts of time exploring both cagemates during training. Table 2 describes average duration spent investigating cagemates during training of young adult (virgin, nulliparous) and middle-aged (retired breeder, multiparous) adult male and female mice. Of note, mice spend considerably more time investigating cagemates during training than is observed with objects described in the previous section, irrespective of age or sex.

Condition	Average Total Time Spent Exploring Cagemates During Training (seconds)
Young Female	57.7
Young Male	55.1
Middle-aged Female	58.4
Middle-Aged Male	56.3

Table 2. Time spent by young and middle-aged male and female mice exploring cagemates as training stimuli in a modified version of the object recognition task.

Rodents are then tested after a four hour retention trial. During testing, one cagemate is replaced with a novel conspecific. A typical process is utilized to assess performance in this version of the object recognition task. That is, the duration spent exploring the novel conspecific versus familiar cagemate is compared. It is calculated as a percentage of total time spent exploring both conspecifics during testing to take into account differences between subjects in exploration of the stimuli during this trial. Chance levels of performance in this task are 50% of time spent exploring the novel conspecific during testing and improved performance in this task is described as more than 50% time spent exploring the novel conspecific in this task.

A pilot study using this protocol was conducted. Performance of young, nulliparous (virgin) male and female mice to middle-aged, multiparous (retired breeders) was compared, and results are depicted in Figure 2. We found that males outperformed females (in diestrus with low endogenous levels of estrogens and progestogens). Performance of young and middle-aged males was similar, but performance of females with extensive breeding history was improved compared to their young, virgin counterparts. These data demonstrate that conspecifics may be used as socially-relevant stimuli to investigate hormonal effects for learning and memory processes. Thus, substituting novel and familiar cagemates as stimuli in an object recognition task may be a means to investigate neurobiological mechanisms underlying learning of socially-relevant stimuli.

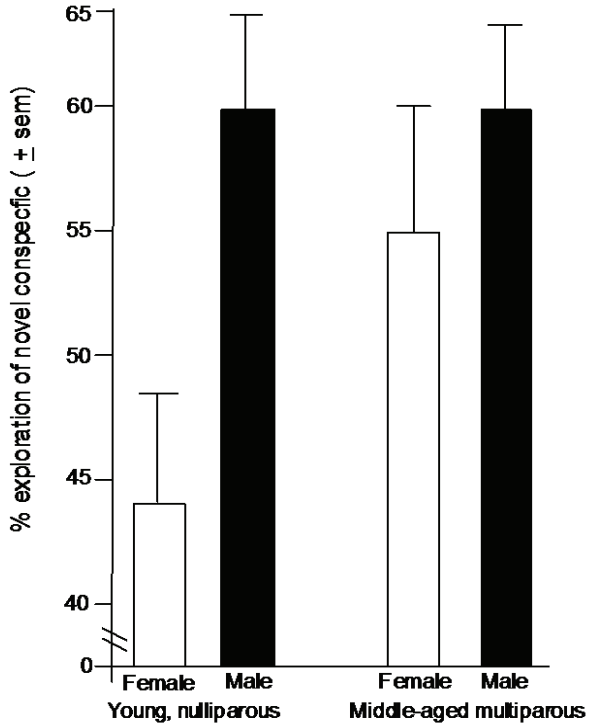

Fig. 2. Cognitive performance of young, nulliparous and middle-aged multiparous female and male mice in the object recognition task using conspecifics as target stimuli.

9. Advantages of using the object recognition task to study cognitive performance across the lifespan

Many aspects of the object recognition task are advantageous to conducting the types of aging and hormone studies described above, as well as studies investigating brain targets and mechanisms underlying these processes. The object recognition task does not require pre-training as it measures spontaneous behavior, exploiting the innate proclivity of rodents to explore novel stimuli. This is a one-trial learning task that does not require multiple, or lengthy, training sessions. This is advantageous to studies of hormonal effects because of the cyclical nature of hormones and we have found that it is important to train and test rodents in the same hormonal state to be able to discriminate the enhancing effects of hormones in object recognition and other tasks (Frye, 1995; Rhodes and Frye, 2004; Walf et al., 2006). As well, object recognition does not rely upon explicit reinforcement with rewarding or noxious stimuli so motivational aspects during training can be minimized. It is advantageous that the target stimuli in this task are not food-based or aversive. This is important in studies of hormones and aging because hormones can influence responses to aversive stimuli (e.g. sensitivities to footshock; Drury & Gold, 1978; Hennessy et al., 1977), as well as food intake (Bell & Zucker, 1971; Frye et al., 1992; Tarttelin & Gorski, 1973). Object recognition is not considered a task that promotes high levels of stress or arousal (Ennaceur & Delacour, 1998). This is advantageous to studies of aging and hormones because hormones alter general arousal (Pfaff et al., 2008). Furthermore, there are interactions of the hypothalamic-pituitary-adrenal and –gonadal axes to influence behavioral responses (reviewed in Frye, 2009; Solomon & Herman, 2009). As such, interpretations of effects may be more straightforward in the object recognition task, in comparison to tasks utilizing aversive stimuli and/or those that influence arousal and stress responding. Another major advantage to using the object recognition task to determine the effects and mechanisms of neuromodulators, such as hormones, is that there is little test-decay in this task when different objects, or conspecifics, are used as target familiar and novel stimuli. This is true as long as there are intervals (days to weeks) between assessments and different objects are utilized (Mumby et al., 2002a). This may be one of the most important factors justifying its use in aging and hormone research. Repeat testing allows for longitudinal studies across the lifespan as well as within-subjects assessments across different natural hormonal milieu (i.e. pregnancy; Paris & Frye, 2008). Thus, there are clear advantages to using the object recognition task to assess the role of hormones across the lifespan.

10. Conclusion

The object recognition task is widely-used to assess non-spatial working, declarative memory task which relies upon a functioning cortex and hippocampus. The typical methods (training, retention interval, and testing) used by our laboratory and others were reviewed with focused consideration on how to use the object recognition task to assess the role and mechanisms of hormones, throughout the lifespan. In addition, there are subjects' variables (e.g. species, strain) that need to be considered in designing experiments and interpreting results using the object recognition task. Another major consideration is the nature and complexity of target stimuli utilized in the object recognition task. The use of objects in object recognition, and findings with regard to aging and hormone studies using the object recognition methods described, were reviewed. Furthermore, a modification to the object

recognition protocol using socially-relevant conspecifics, instead of non-socially-relevant objects, was described. Representative data obtained, when using this task with conspecifics to assess learning and memory processes in rodent models, was discussed. Several advantages to using the object recognition task were discussed with respect to training requirements and interpretations. As well, the major advantage to using the object recognition task to determine the effects and mechanisms of neuromodulators, such as hormones, is the absence of test-decay when different target stimuli are used was discussed. This allows for within-subjects designs and longitudinal assessments, which can be particularly important for studies of changes in natural hormonal milieu with aging. Thus, the object recognition task may be particularly suited to assess changes across the lifespan in cognitive performance to reveal mechanisms in the cortex and hippocampus.

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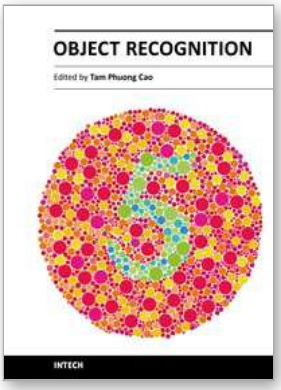

Object Recognition

Edited by Dr. Tam Phuong Cao

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Vision-based object recognition tasks are very familiar in our everyday activities, such as driving our car in the correct lane. We do these tasks effortlessly in real-time. In the last decades, with the advancement of computer technology, researchers and application developers are trying to mimic the humanâ€™s capability of visually recognising. Such capability will allow machine to free human from boring or dangerous jobs.

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