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Examination of Sexually Dimorphic Behavior on the Novel-Image Novel-Location Recognition Memory Test

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Abstract

Objectives: Sex differences in object location memory favoring females appear to be a replicable phenomenon but may also depend on the task demands. This investigation evaluated if females outperformed males at both a short (immediate) and long (half-hour) interval between the learn and test condition using a recently developed version of the Novel-Image Novel-Location (NINL) test (Piper *et al.* 2011, Physiology & Behavior, 103, 513 - 522). **Methods:** Young-adults (N = 184) completed a standardized handedness inventory and the NINL. **Results:** Participants assigned to the Immediate and Delayed conditions did not differ in age, sex, or handedness. The NINL total score was higher among females at the Immediate, but not Delayed, interval. However, within the Delayed condition, females excelled at correctly identifying the unchanged items with a similar pattern for the Novel-Location (NL) scale. **Conclusions:** These findings are consistent with the view that sexually dimorphic performance favoring females in neurocognitive function can also extend to tasks that have a spatial component.

Keywords: Apolipoprotein E, Female, Learning, Memory

1. Introduction

Our understanding of the domains where there are sex differences in neurobehavioral function continues to be expanded and refined [1]. The standard view that women excel at verbal tasks and men at spatial tasks is likely an oversimplification as a female advantage has also been identified on selected measures that also have a spatial component [2]. One area that has repeatedly been shown as sensitive to sex differences is the Object Location Memory (OLM) in which participants are first instructed to learn a large set of common objects (e.g. an umbrella) and, on subsequent trials, identify which pairs of objects have switched positions [3,4]. The OLM has also been expanded to include a condition in which an object is moved to a formerly empty space. In contrast to the location-exchange, the location-shift condition did not show a female advantage [5], although see [6].

The Novel-Image Novel-Location (NINL) test contains some conceptual similarities to the OLM but is procedurally different as it is based on the rodent object-recognition paradigm [7,8]. Participants view sets of three unfamiliar pictures (learn images) and are subsequently asked to identify whether one of the three pictures has been replaced (the Novel-Image or NI condition) or relocated (the Novel-Location or NL condition [9]. In a broadly aged sample (6 to 86), we recently identified a significant female advantage on both NI and NL when the test was conducted immediately after learning the images [10]. Interestingly, the retention interval may be a key element in the detection of sex differences [11]. Therefore, the primary objective of the present report was to attempt to replicate and extend upon the sex difference observed in NINL using a more homogenous aged sample (young-adults) and at different intervals. As the NINL instrument [10] has undergone some modifications relative to an earlier version [9], a secondary goal is to complete a more refined analysis of each item and the correspondence with the total NINL scores.

2. Methods

2.1. Procedures

The Institutional Review Board at Willamette University

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approved all procedures. Subjects consisted of college students from a small private school receiving credit for experimental participation. Participants completed a questionnaire that queried about their age and sex and were asked which hand they used to complete activities. A Laterality Index was computed by adding up the number of activities (e.g. writing, drawing), completed with each hand and using the formula [(R – L)/(R + L)]* 100 [12]. A saliva sample was obtained for apolipoprotein E genotyping according to procedures outlined in [13].

The NINL, version 0.21, was an extension of the previous NINL test [9]. The differences compared to version 0.1 were: 1) stimuli were imported into a slide show in Microsoft Power Point to precisely regulate the display time; 2) more detailed instructions and a practice trial so that the test could be more readily completed in a group setting; 3) the number of pictures was doubled to eighty with high resolution neutral images (e.g. a dustpan) from the International Affective Picture System [14]; and 4) the location of the changed item balanced across the four potential quadrants. Each slide was shown for 8.0 sec during the learn phase. Either directly or approximately one-half hour after viewing these slides (henceforth referred to as Immediate or Delayed), testing commenced (eight NI, eight NL, and eight No-Change or NC). Participants in the Delayed condition completed other distracter tasks during the interval. The quadrant of the NI or NL was dispersed across the four quadrants with item type (NI, NL, or NC) staggered within the Learn and Test sets (see **Table 1** for further details). Scoring consisted of 0 - 3 points per NI/NL item with zero points awarded if the subject could not identify if a change had occurred, one point for correctly identifying that a change had happened, two points for also recognizing the change type (NI or NL), and three points for the previous plus accurately identifying the quadrant of the change. Correct identification of a NC item resulted in three points.

2.2. Data-Analysis

All statistics were performed using SPSS, version 16.0 (SPSS Inc., Chicago, IL) with an alpha level of 0.05. Pearson correlations were completed for each item score with the corresponding scale (NI, NL, or NC) and with the NINL total (NI + NL + NC). Mean data is presented with the SEM. Cohen's *d* was determined for group differences with 0.20 interpreted as small, 0.50 as medium, and 0.80 as a large effect size.

3. Results

The Immediate and Delayed groups did not differ based on age, sex, or handedness (**Table 2**). **Table 3** shows the item to scale and item to total correlations for the 24-item NINL instrument were all positive and significant. The item to scale associations were similar for the NC (Min = 0.35 to Max = 0.63), NI (0.32 to 0.61), and NL (0.30 to 0.59) scales. The item to total NINL score correlations were generally homogenous for NC (0.21 to 0.52) and NI (0.26 to 0.51). Interestingly, the first NINL item also had the lowest correlation ($r = 0.17$) but values for the remaining seven NL items were higher ($r = 0.29$ to 0.43). Further, the NC scale was moderately correlated with NI ($r(167) = 0.39$, $p < 0.0005$) and NL ($r(169) = 0.39$, $p < 0.0005$) and NI and NL showed a similar association ($r(163) = 0.37$, $p < 0.0005$).

Figure 1(a) shows that total NINL performance differed by both sex and retention interval. Females had higher scores than males in the Immediate ($d = 0.59$) but not Delayed condition. Females scored lower at the long interval relative to the Immediate ($d = 0.47$). Further analyses for each scale (**Figure 1(b)**) shows that sex, NINL difficulty, and task demands each determined recognition memory. Within the Immediate, males were

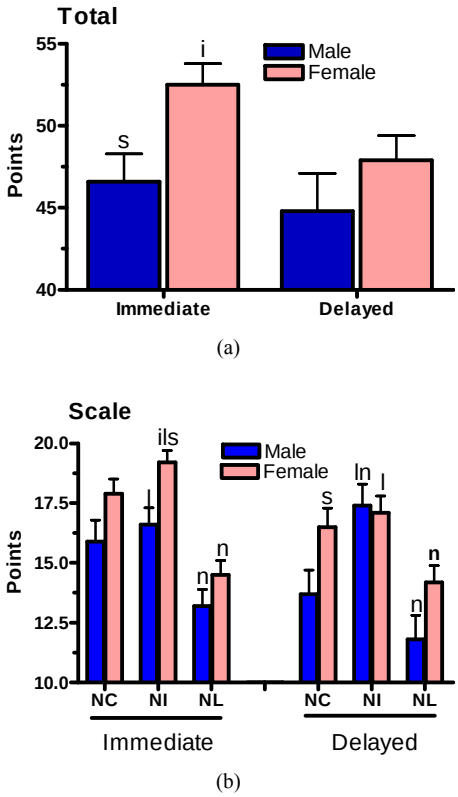

Figure 1. Novel-Image Novel-Location performance total (a) and for each scale (b) by sex and retention interval. NC: No-Change; NI: Novel-Image; NL: Novel-Location; $^{s}p < 0.05$ sex difference within an interval; $^{i}p < 0.05$ interval differences versus delayed; $^{n}p < 0.05$ scale difference versus NC; $^{l}p < 0.05$ scale difference versus NL.

significantly lower on NI (d = 0.59) but did not differ significantly on NC ($t(104) = 1.94$, $p = 0.055$, $d = 0.38$) from females. In the Delayed, females outperformed males on NC (d = 0.50) and showed a similar tendency for NL ($t(72) = 1.99$, $p = 0.051$, $d = 0.46$). The NL was lower than both NC and NI independent of sex and interval.

4. Discussion

This report contributes to a very large and evolving literature on sex differences in neurocognitive function [1]. Clearly, the identification and direction of group differences based on sex depends on the sample size and nature of the task employed including how it is scored [2]. Males outperform females on the mental rotation test [4,15] and exhibit almost equally large group differences on spatial navigation measures [9,10,16]. In contrast, less is definitely known about other domains. Although the NINL total was elevated among females only at the immediate interval, examination of each scale revealed a moderate effect size ($d \approx 0.50$) for NI at the shorter period and on both NL and identifying the unchanged items at the longer period. This finding is broadly concordant with our previous study with a lifespan sample [10] and in line with a prior investigation with the OLM in which college aged females scored higher than males on identifying drawings of new objects (d = 0.46) and location exchanges (d = 0.44, [3]. However, in the location shift of the OLM (the condition most similar to the NL), prior results have been contradictory with some [6], but not all [5], investigations identifying better performance among women.

In the OLM, participants view the original learn array and then, during repeated testing, may view elements of this same array again which makes these conditions difficult to compare directly. As Levy *et al.* [6] noted, this design could also introduce proactive interference. An advantage of the NINL is that this instrument has different learn sets that are subsequently tested in the NI, NL, or NC. Rodents find the location test much more challenging than the novel-object test [7]. Similarly, human participants did significantly less well on NL compared to NI at both intervals indicating that this is a robust phenomenon across species. The neural substrates that mediate these task specific processes have not yet been characterized on NINL but investigations with rats have shown that lesions of the perirhinal cortex impact object recognition memory [8]. It is tempting to speculate that location recognition would be more dependent on the dorsal stream (*i.e.* where) and image recognition on the dorsal ventral stream (*i.e.* what) structures although the integration of both occipto-parietal and occipito-temporal regions may also be essential [17].

The interval between the learn and test phases is a key element in task difficulty [18]. Adult female rats showed object-recognition at intervals (1 - 3 hours) that males could not. Conversely, males were capable of identifying when an object had been moved to a new position at several intervals that were too difficult for females [11]. A potential limitation is that only two intervals were examined in the present study. A slightly longer (1 hour) delay was evaluated during pilot testing and found to be unfeasible for this participant population. A comparison between an immediate and a 24-hour retention period produced the anticipated reduction at the longer-interval in a separate all-female sample [Thornburg & Murphy, unpublished observations] but a multi-interval examination of sex differences should be a topic for further research.

A fundamental characteristic of any cognitive test is the type of the material to be learned. In addition to other caveats, this factor may also limit the generalizability between rodent and human recognition memory tests. Investigators utilizing laboratory animals can quite easily use objects that their subjects have never before experienced [18]. This frequently takes the form of children's toys [7] or commonplace household objects [19]. The NINL learn set (**Table 1**) consists of pictures from a database maintained for research purposes [14]. However, the images are of objects or scenes that can readily be labeled. Although the magnitude of sex differences in generalized verbal abilities is a matter of some contention [20], we suspect that females were more likely to employ verbal strategies to facilitate a deeper encoding of the image sets. Therefore, it is quite interesting that when abstract line drawings (which would limit the potential to label) are used as stimuli, there is conflicting data whether a female advantage is still detected [21-23]. Further, the Design Memory subtest of the Weschsler Memory Scale-IV includes a topographically more complicated NL type element with geometric shapes and abstract stimuli and sex differences are very minimal (personal communication from JM Laurer).

There are several future directions that may be worthy of some consideration for investigators interested in individual differences in NINL performance. A fascinating report determined that homosexual males substantially outperformed heterosexual males on Object-Location Memory [24]. Additional study with a more ethnically and socioeconomically diverse sample in which other information (e.g. sexual orientation, IQ, EEG) is obtained may prove fruitful as these factors could contribute to the present neurobehavioral profile.

Overall, there are clear sex differences on NINL which, when analyzed at the level of each scale, are independent

Table 1. Images for the Novel-Image, Novel-Location test, Learn (L) and Test. Image numbers (in parentheses) are from the International Affective Picture System ([14]). Quadrants are north-west (NW), north-east (NE), south-east (SE) and south-west (SW) with empty quadrants depicted by a dash (-). Type is Novel Image (NI), Novel Location (NL) or No Change (NC).

Learn					Test			
Slide	Quadrant				Slide			
#	NW	NE	SW	SE	#	Basis	Type	Description
L01	-	outlet (6150)	mushroom (5531)	train (7039)	T01	L07	NL	car (8531) to NE
L02	dust pan (7040)	pocket watch (7190)	-	towel (7002)	T02	L16	NC	
L03	coffee cup (7057)	dark cloud (5594)	cube (7185)	-	T03	L04	NI	clock (7211) at SE
L04	hammer (7034)	book (7090)	-	abstract painting (7830)	T04	L11	NI	crimps (7056) at NW
L05	yard (5130)	-	shoes (7031)	woven basket (7010)	T05	L10	NC	
L06	set table (5849)	canyon (5661)	-	row boats (5390)	T06	L24	NL	pins (7052) to NW
L07	hair dryer (7050)	-	convertible car (8531)	fan (7020)	T07	L05	NC	
L08	-	leaves (5750)	fork (7080)	parking lot (7595)	T08	L17	NI	stool (7025) at SW
L09	fireworks (5480)	-	unlit light bulb (7055)	waste can (7060)	T09	L18	NC	
L10	-	satellites (5471)	blue cup (7009)	headlight (7095)	T10	L02	NL	pocket (7190) to SW
L11	rolling pin (7000)	bus (7140)	tiger lilly (5030)	-	T11	L08	NI	clothes (7242) at SW
L12	drill (7043)	latch (7059)	-	Native Amer pattern (7179)	T12	L03	NL	cube (7185) to SE
L13	blue door (5731)	bridge (7547)	pink flower(1604)	-	T13	L06	NC	
L14	-	power lines (9080)	spoon (7004)	abstract painting (7161)	T14	L15	NL	bulb (7170) to SE
L15	lit bulb (7170)	ship (5395)	large baskets (7041)	-	T15	L19	NC	
L16	orchid (5010)	-	leaves (5740)	empty pool (9360)	T16	L09	NI	rack (7217) at NW
L17	earth (5890)	mountain top (5660)	airplane (7620)	-	T17	L23	NI	field (5250) at NW
L18	-	white bowl (7006)	tissue (7950)	sports car (8510)	T18	L22	NC	
L19	clear glass (7035)	hydrant (7100)	gold bars (8500)	-	T19	L13	NC	
L20	-	ferris wheel (7508)	orange flower (5020)	file cabinet (7705)	T20	L20	NL	wheel (7508) to NW
L21	dumbells (7042)	-	lamp (7175)	yellow sail-boat (8210)	T21	L21	NI	universe (5300) at SE
L22	shoes (7038)	scarf (7205)	building (7491)	-	T22	L12	NL	Native (7179) to SW
L23	flowers (5000)	semi-truck (7130)	-	freeway (7560)	T23	L14	NI	plate (7233) at NE
L24	-	clothes pins (7052)	umbrella (7150)	snow day (5635)	T24	L01	NL	train (7039) at NE

of the retention interval. The cognitive (e.g. attention or encoding) or biological (e.g. distinct neural substrates) mechanisms responsible for the female advantage on this and other similar tasks will be the subject of additional study. A strength of the NINL is that this procedure is based on the rodent object recognition test which may aid in translating the substantial knowledge base from rodents to humans.

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Table 2. Participant characteristics by Novel-Image Novel-Location retention interval. apoE4: Apoliprotein E4 allele.

	Immediate (N = 106)	Delay (N = 78)
Age	19.0 (0.1)	18.7 (0.1)
Sex (% Female)	61.3%	53.8%
apoE4+	21.7%	17.9%
Laterality Index	0.23 (0.06)	0.33 (0.07)

Table 3. Pearson correlations for each item with the scale and total score (in parentheses) for the Novel-Image Novel-Location test. The *p* value was ≤ 0.001 for all correlations except [a]*p* < 0.05.

Item	No Change	Novel-Image	Novel-Location
1	0.51 (0.41)	0.61 (0.46)	0.30 (0.17[a])
2	0.50 (0.40)	0.32 (0.33)	0.46 (0.43)
3	0.45 (0.21)	0.39 (0.25)	0.59 (0.41)
4	0.48 (0.36)	0.50 (0.31)	0.53 (0.35)
5	0.57 (0.39)	0.42 (0.26)	0.51 (0.38)
6	0.35 (0.28)	0.58 (0.51)	0.51 (0.37)
7	0.63 (0.52)	0.42 (0.32)	0.50 (0.43)
8	0.41 (0.35)	0.60 (0.47)	0.47 (0.29)

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