Supplementary Online Material to "Flexible categorisation of hands and tools in prosthesis users"

Methods

Participants

Twelve individuals with acquired unilateral upper limb amputation (mean age (SD) = 43.1 (12.5), range 24-59; mean (SD) age at limb loss = 28.6 (9.6), range 17-45; 2 with absent right hand; 3 women), 12 individuals with congenital unilateral limb absence (mean age (SD) = 42.4 (12.9), range 25-61; 2 with absent right hand; 6 females) and 21 able-bodied controls (mean age (SD) = 41.1 (11.8); 7 left-handed; 9 females) were included in this study (see Table S1 for demographic and clinical details of one-handed participants). Data for 3 additional participants was collected but were discarded from the analysis as outliers (see Data analysis). Sample size was determined a priori based on the paper by Xu, Lauwereyns, & Iramina (2011) which inspired our experimental design. The main effect of interest was the prime*target interaction (congruency effect). Since an exact power analysis could not be performed based on the information available in that study, a similar number of participants was recruited for the one-handed and control groups. Recruitment was carried out through Opcare (the largest provider of prosthetic services to the National Health Services in the UK) in accordance with Oxford University's Medical Sciences inter-divisional research ethics committee (Ref: MSD-IDREC-C2-2014-003). Written informed consent and consent to publish was obtained from participants prior to study participation in accordance with ethical standards set out by the Declaration of Helsinki (1964).

ID	Group	Gender	Age	Age at limb loss	Side	Level	Cosm. freq.	Mech. freq.	Myo. freq.	MAL(P)	Pros. usage	PS	PLP
C1	Congenital	Female	52	0	R	TR	5	1	0	0.15	1.37	0	0
C2	Congenital	Male	52	0	L	WD	0	3	0	0.04	-0.28	0	0
C3	Congenital	Male	49	0	L	TH	1	4	0	0.28	1.48	0	0
C4	Congenital	Female	28	0	L	WD	0	0	0	0	-2.11	0	0
C5	Congenital	Female	27	0	L	TR	5	0	0	0.54	3.34	0	0
C6	Congenital	Male	60	0	L	WD	2	0	0	0.06	-0.72	0	0
C7	Congenital	Female	34	0	R	TR	5	0	0	0.46	2.93	0	0
C8	Congenital	Female	61	0	L	WD	5	0	0	0.54	3.34	0	0
C9	Congenital	Male	25	0	L	TR	1	0	5	0.59	3.59	0	0
C10	Congenital	Male	34	0	L	WD	0	0	3	0.11	0.08	0	0
C11	Congenital	Male	38	0	L	TR	0	2	1	0	-1.02	0	0
C12	Congenital	Female	49	0	L	WD	1	0	0	0	-1.57	0	0
A1	Amputee	Male	59	40	L	TH	0	0	0	0	-2.11	48	23
A2	Amputee	Male	58	27	L	TH	5	2	0	0.04	0.81	35	45
A3	Amputee	Male	53	28	L	TR	3	5	0	0.24	1.82	10	20
A4	Amputee	Male	48	17	L	TH	2	2	0	0	-1.02	100	25
A5	Amputee	Female	46	38	L	TR	0	0	0	0	-2.11	90	94
A6	Amputee	Female	24	18	R	TR	0	0	0	0	-2.11	100	45
A7	Amputee	Male	49	37	L	TH	1	0	0	0	-1.57	20	27
A8	Amputee	Female	50	45	L	TH	0	2	0	0	-1.02	90	70
A9	Amputee	Male	29	24	L	SD	0	0	2	0.09	-0.57	40	18
A10	Amputee	Male	25	18	L	WD	0	2	0	0	-1.02	100	30
A11	Amputee	Male	45	20	R	TR	2	0	0	0.11	-0.47	50	10
A12	Amputee	Male	32	31	L	TH	0	2	0	0	-1.02	100	50

Table S1: Demographic details of one-handers with congenital (C) and acquired (A) limb loss. Side = side of limb loss: L – left, R – right. Level of limb loss: the level at which the residual arm ends; SD – shoulder disarticulation, TH – transhumeral limb loss, TR – transradial limb loss, WD – wrist disarticulation. Cosm. = Cosmetic prosthesis, Mech. = Mechanical prosthesis, Myo. = Myoelectric prosthesis. Freq. = frequency of prosthesis usage; 0 – never, 1 – rarely, 2 – occasionally, 3 – daily (<4 hours), 4 – daily (4-8 hours), 5 – daily (>8 hours). MAL(P)= Motor Activity Log (Prosthesis) score: range 0-1. Pros. usage = prosthesis usage; compound score that contains both frequency of wearing and incorporation of the prosthesis in daily activities, calculated as the sum of standardised (Z-transformed) maximum frequency and MAL(P) scores. PS = phantom sensations. PLP = phantom limb pain. Amputees rated the intensities of painful and non-painful phantom sensations using a 0-100 scale as well as the frequency of these experiences (1 – all the time, 2 – daily, 3 – weekly, 4 – several times per month, 5 – once or less per month). Chronic PS and PLP was calculated by dividing intensity by frequency.

<u>Artificial limb usage measurements</u>

The use of the prosthetic limb was initially assessed using a revised version of the Motor Activity Log as described and validated by Makin et al. (2013). In brief, participants were requested to rate how frequently they incorporate their prosthesis in an inventory of 27 daily activities, requiring varying degrees of motor control. Each item was scored (0: “never”, 1:

“sometimes”, 2: “very often”). The sum of all items was divided by the highest possible score, such that individuals were rated on a scale between 0 to 1. As it is possible that participants wear the prosthesis for other purposes than stated in the inventory (e.g. cosmetic purposes), we additionally asked participants to rate how much time they typically spend wearing their prosthesis (0 - never, 1 - rarely, 2 -occasionally, 3 - daily [<4 hours], 4 - daily [4-8 hours], 5 - daily [>8 hours]). Both ratings were standardised using a Z-transform and summed to create a usage score that included both wear time and incorporation of the prosthesis in day-to-day activities.

<u>Stimuli</u>

Tool and hand images were taken from an online database. Twenty hand-held tools, purposed to elongate the arm (e.g. hammer, spatula, see Fig. S1) were selected for the tool category, of which ten were used as primes and ten as targets. Twenty pictures of a hand were selected for the hand category (both with and without an arm to account for participants’ varying levels of limb loss), of which ten were used as primes and ten as target stimuli. Pictures of each one-hander’s prosthetic limb were taken by the experimenters prior to the testing session from 7 different angles (both first and third person perspectives). One-handers were presented with pictures of their own prostheses. Three amputees and two congenital one-handers who did not bring a prosthesis to the study, and all controls, were presented with prosthesis images of a randomly selected other participant’s prosthesis. Hand and prosthesis images were matched (lateralised) to one-handers’ missing hand side and to the nondominant hand side in controls, so that, for example, a left-side amputee would be presented with left hands and “left-hand” prostheses. In aligning the stimuli with the missing hand in one-handers, we intended to match the hand images with the prosthesis laterality. In the control group, the non-dominant hand was used, based on the rationale that the intact hand is conceptually more similar to the dominant hand, as it fulfils all the functions of the dominant hand in daily life. However, given the diversity of the hand pictures used here (Figure S1a) and their short presentation time

(32ms; see below), and considering that laterality judgements in one-handers take seconds (Nico et al., 2004), laterality might not have been a salient feature in our study

Stimuli were edited using Adobe Photoshop, such that each stimulus had its background removed, was converted to greyscale, was placed on an equiluminant grey background, approximately normalised for size, and was overlaid with a fixation point. A set of "scrambled" images was created by applying a diffeomorphic transformation to the experimental stimuli, which maintains the typology of the original image (Stojanski & Cusack, 2013). This type of image scrambling better preserves the basic visual properties of the original image relative to other methods of image scrambling. We applied 20 iterative steps of the transformation to ensure images were unrecognisable. Example stimuli of each category can be found in Fig. S1.

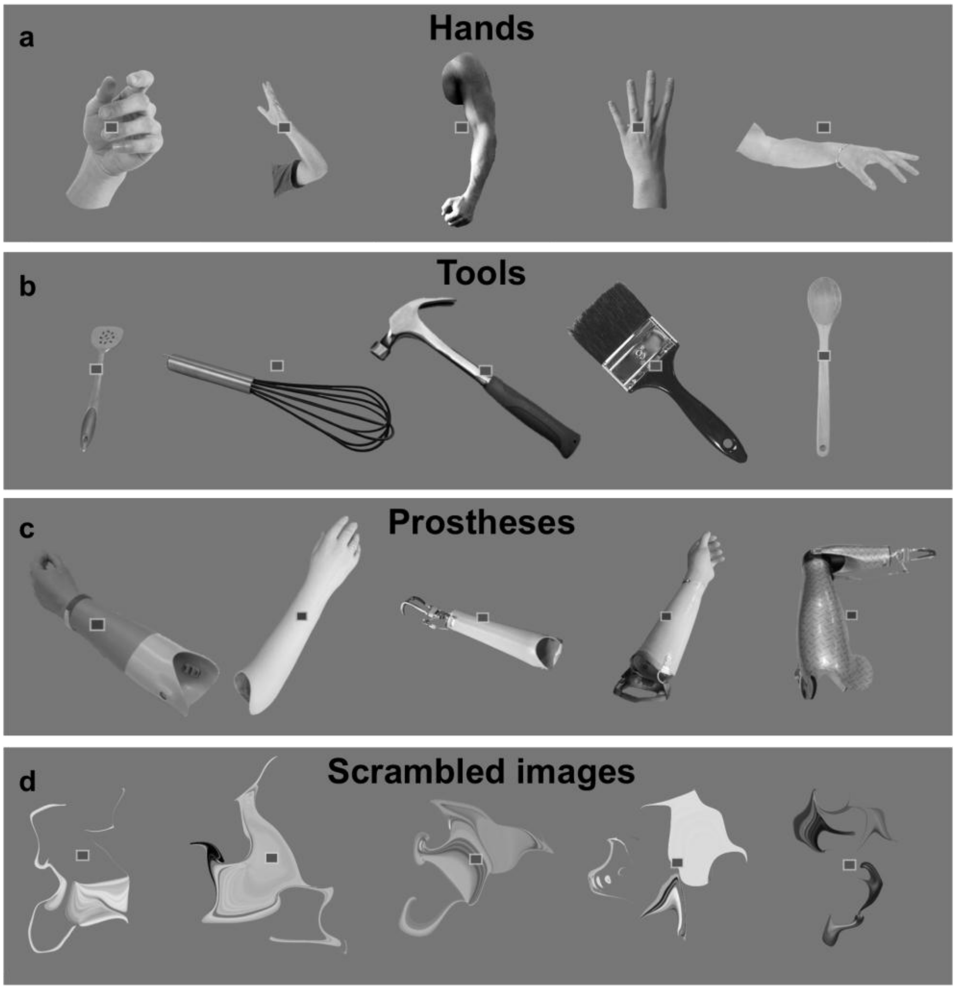

Fig S1. Examples of experimental stimuli. (a) Images of upper limbs, including hands shown alone and with the arm shown. (b) Manual tools that extend the hand and arm. (c) Pictures of the participants' own prostheses were taken by the experimenters prior to the testing session and used as stimuli. Participants were presented with their own prosthesis if possible. Hand and prosthesis images were matched to the side of the participants' missing or nondominant hand (e.g. a left-side amputee was presented with left hands and left prostheses). (d) A subset of the experimental stimuli underwent a diffeomorphic transformation to create a set of "scrambled" images that were unrecognisable but maintained the basic visual properties of the original images. Scrambled items were used as neutral primes in the baseline trials.

Experimental procedure

The experiment was designed and performed using Presentation software (www.neurobs.com, version 16.3). Participants received verbal instructions prior to the task. During the experiment, participants were seated comfortably in front of a laptop computer while wearing a lapel microphone. Before starting the experiment participants were asked to pronounce the words "hand" and "tool" a few times at a comfortable speaking volume while the experimenter set the recording thresholds in order to minimise false positives and misses. At the beginning of each experimental block, participants pressed the space bar to start or continue the experiment. Each trial consisted of the presentation of a prime stimulus followed by a target stimulus. Trials started with a fixation point presented in the centre of the screen for 800 ms. The prime stimulus (hand, tool, or prosthesis) was presented for 32 ms. 600 ms after prime onset the target was presented for 32 ms (Xu, Lauwereyns, & Iramina, 2011). On each trial, participants were asked to make a speeded forced-choice verbal response whether the target stimulus was a hand or a tool by verbally responding "hand" or "tool". Participants were explicitly instructed to ignore the first image and only respond to the second image, and to respond as quickly as they can while keeping errors to a minimum. A lapel microphone recorded the voice onsets as reaction times (RTs) and audio files were recorded by the experimental software for offline analysis. After each response the experimenter recorded the nature of the response (hand or tool) by pressing one of two keys on a remote keyboard, which triggered the start of the next trial. Note that the registration of the response by the experimenter introduced temporal jitter between two consecutive trials.

A total of 40 baseline trials, containing neutral (scrambled) primes, preceded the experimental trials. In the main experiment, six experimental conditions were randomly intermixed: hand-hand; tool-hand; prosthesis-hand; hand-tool; tool-tool; prosthesis-tool. Images for primes and targets were paired in advance such that each stimulus combination was shown no more than once throughout the experiment. The experiment consisted of four blocks with self-paced breaks between blocks. Each block consisted of 60 trials which were randomly presented, giving 40 trials in each experimental condition. Each prime image exemplar (hand, tool, prosthesis) was presented eight times over the course of the experiment. Each target image exemplar (hand, tool; different from prime stimuli) was presented 12 times over the course of the experiment.

Data analysis

Preprocessing

All audio recordings were inspected by a naïve experimenter to discard trials where the recording was triggered by noise. Noise trials (5.9%) and erroneous responses (0.7%) were discarded from the analysis. Trials were additionally discarded if the sound onset was shorter than 300 ms so as to remove additional noise, which led to the removal of an additional 0.3% of trials, or exceeded the mean of the respective condition by 3 standard deviations (1.8%). The resulting trials were averaged for each experimental condition within each participant for group analyses. Three additional congenital one-handers were excluded from the group data analysis as they displayed mean RTs exceeding the upper quartile by more than 1.5 times the inter-quartile range (Tukey fences; Tukey, 1970).

Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics for Macintosh Version 22.0. In tests where Mauchly's test of sphericity was violated, a Greenhouse-Geisser correction was applied. We performed a mixed-level analysis of variance (ANOVA) with the within-participant factors prime (hand, tool, prosthesis) and target (hand, tool), and a between-participant factor group (one-handers, controls). To assess the effect of the primes, we further compared RTs of congruent to incongruent trials. We calculated the hand/tool congruency effect as the sum of the differences in RTs [(hand-hand & tool-tool) – (tool-hand & hand-tool)]. This calculation reflects the interaction between the prime and target categories, while considering the direction of the priming effect observed in controls, showing slowing of RTs for same-category versus different-category trials (see below). Note that for the purpose of this study, the direction of the interaction (positive or negative priming) was irrelevant. To assess the predicted relationship between the congruency effect and experience with natural and artificial limbs (age at limb loss and prosthesis usage respectively) we performed correlations within the relevant groups; all one-handers were included in the latter, whereas only acquired amputees were included in the latter. To test whether inter-subject variability differed between the groups, we ran the Levene test of homogeneity of variance.

To explore which factors contribute to the effect of a prosthesis prime on hand and tool processing, we tested a range of clinical and experimental variables, which could be directly or indirectly related to the categorisation of prostheses. Based on a priori hypotheses as well as our finding for hand/tool categorical dissociation (see Results), hand-experience could be a driving factor for changes in prosthesis categorisation. We therefore included two measurements of years of experience with or without having a hand: age at limb loss and years since limb loss. Note that by including these two measurements, the participants' age is indirectly included as a potential factor in the model. Secondly, a main candidate for predicting the categorical association of prostheses to hands and/or tools was prosthesis usage, to assess whether experience with an object can drive changes in its categorisation.

The congruency effect was also included, to ascertain whether a general lack of task sensitivity could explain variance in prosthesis-prime trials. Finally, baseline RT was included to account for between-participant differences in RT due to general processing speed differences. The relevant parameters were enetered into a backwards stepwise regression with the criteria: probability of F-to-remove ≥ 0.1, probability of F-to-enter ≤ 0.05.

Results

Mean RTs for each condition and group are shown in Fig. S2. First, we performed a prime(3; hand, tool, prosthesis)*target(2; hand, tool)*group(2; one-handers, controls) mixed-level ANOVA. There was no main effect of prime ($F < 1$) or of group ($F < 1$). We found a main effect of target ($F(1,43)=59.08$, $p<0.001$), with responses to hands being faster than responses to tools, likely due to the different initial phoneme of the verbal response, affecting naming time and voice key recording (Rastle & Davis, 2002). Additionally, we found a significant three-way interaction between prime, target and group (Greenhouse-Geisser corrected $F(1.62,69.82)=3.64$, $p=0.040$), suggesting differences in prime-target interactions across groups. To interpret this three-way interaction, we performed separate prime(3)*target(2) repeated measures ANOVAs in each group, as detailed below. The three-way ANOVA also revealed significant two-way interactions. First, we found an interaction between prime and target (Greenhouse-Geisser corrected $F(1.62,69.82)=5.57$, $p=0.009$), indicating that primes affect processing of the target. Next we found an interaction between prime and group ($F(2,86)=4.11$, $p=0.020$), showing that prime stimuli, regarless of the associated target, affect one-handers and controls differentially. We found no interaction between target and group ($F < 1$).

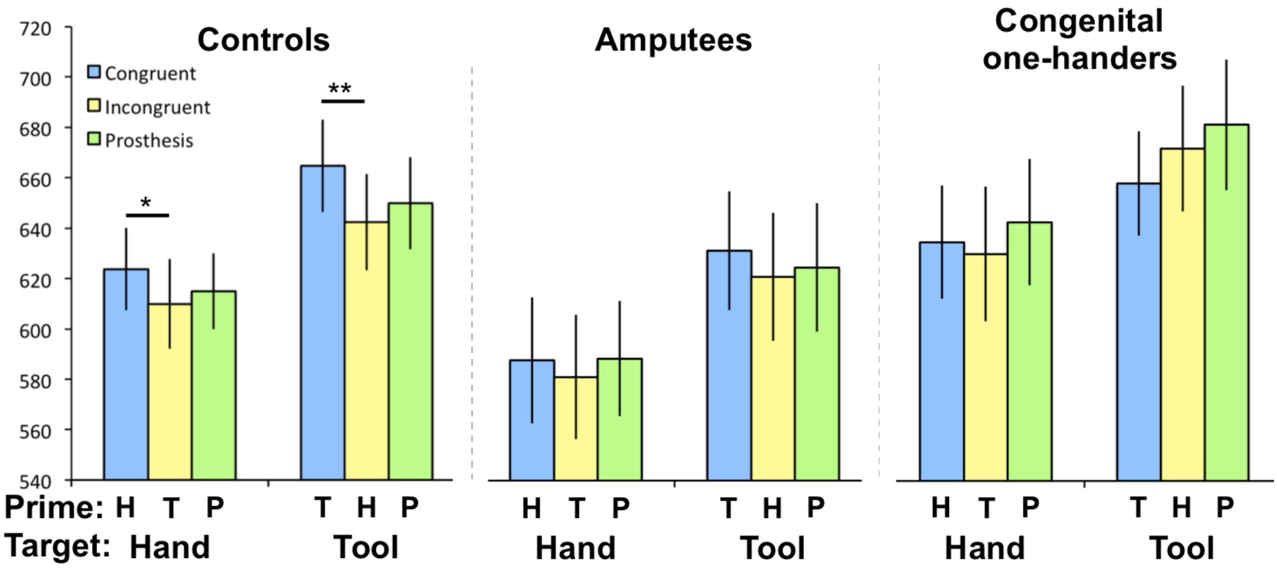

Fig S2. Mean reaction times for each of the six experimental condition in the three study groups (left: controls, middle: amputees, right: congenital one-handers). The prime (H - hand; T – tool; P – prosthesis) and the target (hand, tool) categories comprising each trial are specified in the bottom. Conditions including same-category prime and target (tool-tool and hand-hand) were labeled congruent (blue). Trials including cross-category prime and target (hand-tool and tool-hand) were labeled incongruent (yellow). Trials involving a prosthesis prime with a hand or a tool target were labels prosthesis (green).Across groups there was a main effect of target, with "tool" responses being slower than "hand" responses. Within the control group there was a significant prime*target interaction, with congruent RTs (hand-hand and tool-tool) being slower than incongruent RTs (tool-hand and hand-tool, respectively). The one-handers did not display such a congruency effect. Error bars indicate one standard error of the mean. Asterisks denote significance of paired-samples t-tests; * $p < 0.05$, ** $p < 0.005$.

Controls

Within the control group, we found a significant prime(3; hand, tool, prosthesis)*target(2; hand, tool) interaction ($F(2,40)=8.55$, $p=0.001$). This indicates that different prime stimuli have a differential effect on target processing of hands and tools, and provides the first evidence for the existence of a congruency effect. This was further confirmed using two planned comparisons (paired samples t-tests) between congruent and incongruent RTs, showing differences on both hand trials ($t(20)=2.19$, $p=0.041$, $d=0.175$) and tool trials ($t(20)=3.31$, $p=0.003$, $d=0.261$). For both target types, congruent RTs were slower than incongruent RTs (Fig. S2). This suggests that a same-category prime interferes with the processing time of the subsequent target image, resulting in negative priming. This interpretation is consistent with the long stimulus-onset asynchronies used in the present study (Boy & Sumner, 2010).

Since prosthesis primes could act as congruent, incongruent, or neutral primes on hand and tool trials, we also performed exploratory paired samples t-tests between hand/tool primes and prosthesis primes, and found differences between Tool-Tool and Prosthesis-Tool RT only ($t(20)=2.50$, $p=0.021$, $d=0.177$), however this did not survive correction for multiple comparisons (Bonferroni-corrected $p=0.084$).

One-handers

We did not find a significant prime(3; hand, tool, prosthesis)*target(2; hand, tool) interaction (Greenhouse-Geisser corrected $F < 1$) within the one-handed group. When only considering hand and tool trials, the one-handed group showed no hand/tool congruency effect, as evidenced by the absence of significant differences between RTs on congruent and incongruent trials (hand trials: $t(23)=1.31$, $p=0.204$, $d=0.064$; tool trials: $t(23)=0.25$, $p=0.803$, $d=0.021$). We additionally split the one-handed group into congenital one-handers and amputees. We found no prime(3)*target (2) interaction in either of the one-handed subgroups (amputees: $F(2,22)=1.26$, $p=0.305$; congenital one-handers: Greenhouse-Geisser corrected $F < 1$). The three groups didn't show difference in overall RTs ($F<1$), RTs for hand targets ($F<1$), overall baseline trials ($F<1$), or in baseline trials for hand targets ($F<1$). When considering the congruency effect (i.e. the interaction between prime/target and hands/tools), the three groups did not show significant differences in variance (Levene Statistic = 0.598, $p=0.55$).

One of the main objectives of the study was to determine whether experience with natural and artificial limb usage affects hand/tool dissociation. To test whether hand/tool categorisation can be modulated by the amount of experience with having a hand, we focused on acquired amputees, who lost their hand at a range of 17-45 years of age. We found that prolonged experience with the hand that was later lost resulted in greater categorical dissociation between hands and tools, as indicated by a positive correlation between age at limb loss and the congruency effect ($r(10)$=0.65, p=0.022, Fig. 1b). These findings suggest that the conceptual distinction between hands and tools develops through experience with natural limb usage. Additionally, across the one-handed group, we found a trend towards a negative correlation between prosthesis usage and the congruency effect ($r(22)$=-0.38, p=0.068), such that category-specific priming effects tended to reduce with the regularity of prosthesis usage. However, since this trend was not significant, we refrain from further interpreting this result.

As detailed in the main text, in the final set of analyses we assessed the degree to which prosthesis primes affect responses to hand and tool targets as a function of experience. To explore group differences associated with responses to the prosthesis primes, we ran a one-way ANOVA with the three groups (controls, acquired amputees and congenital one-handers) for prosthesis prime trials (with baseline RT trials as covariates, accounting for between-subject differences that are not specific to the prosthesis prime). We found a significant main effect of group (F(2,21)=0.949, p=0.027), which was further reflected in slower RT for congenital one-handers compared with the controls (F=3.88, p=0.058), but not with the acquired amputees (F=0.81, p=0.377). Given that for hand/tool sequences, conceptual similarity was reflected in slower responses for congruent prime target pairs, slowing of RTs for prosthesis primes can be used to estimate the conceptual similarity between prostheses and hands or tools.

To further explore this effect, we used a backwards regression analysis on RT for hand and tool targets and found that people who lost their hand earlier in life showed a stronger categorical relationship between the prosthesis and hands. This was exemplified by a backwards regression analysis on prosthesis-hand RT using the following predictors: baseline RT, congruency effect size, age at limb loss, years since limb loss and prosthesis usage. The final model for hand-target trials ($F(2,21)=35.08$, $p<0.001$, $R=0.88$, $R^2_{adj}=0.75$) included baseline RT ($\beta=0.78$, $t(23)=7.28$, $p<0.001$) and age at limb loss ($\beta=-0.29$, $t(23)=-2.72$, $p=0.013$). Details of the regression models are shown in Table S2. The partial regression plots for the final model are shown in Fig. S3. Furthermore we found that the conceptual relationship between prostheses and tools was best predicted by prosthesis usage, with those using their prosthesis more showing a greater conceptual similarity between prostheses and tools. This finding was supported by a backwards regression analysis on Prosthesis-Tool-RT. The final model ($F(2,21)=42.48$, $p<0.001$, $R=0.90$, $R^2_{adj}=0.78$) included baseline RT ($\beta=0.83$, $t(23)=8.36$, $p<0.001$) and prosthesis usage ($\beta=0.24$, $t(23)=2.39$, $p=0.026$). Details of the regression models are shown in Table S3. The partial regression plots for the final model are shown in Fig. S4.

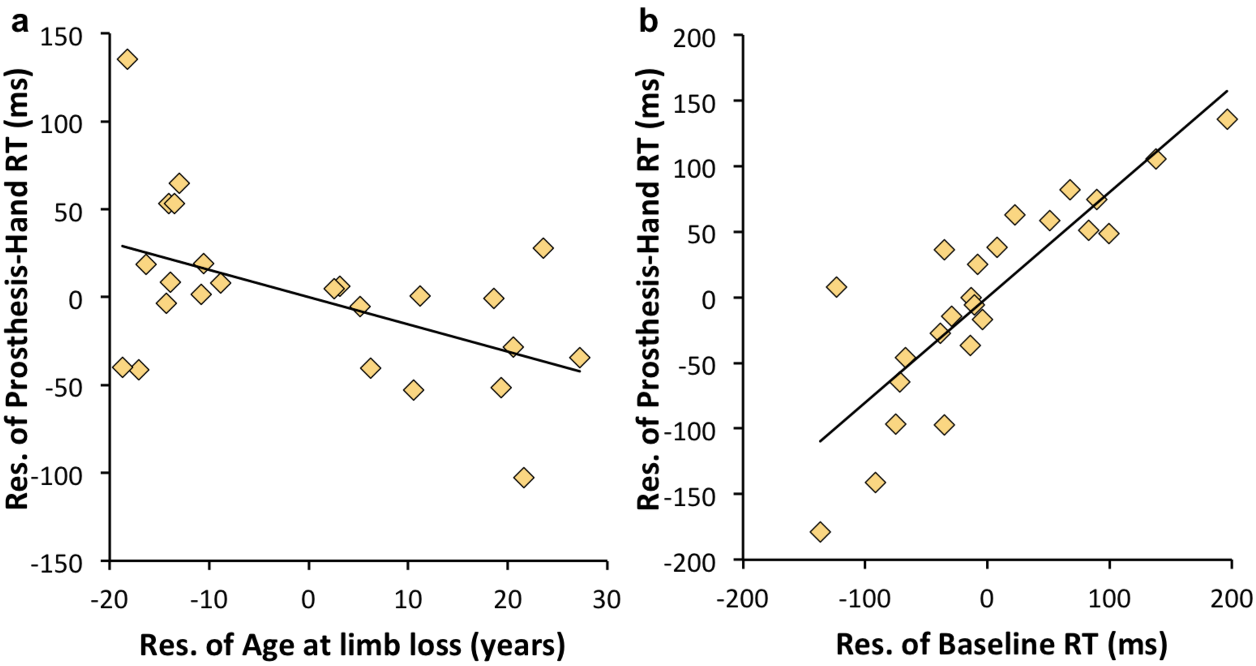

Fig S3. Partial regression plots for the final model of the backwards regression of prosthesis-hand RT. The final model included two predictors: (a) age at limb loss, suggesting that less experience with a natural limb leads to a strengthened categorical relationship between prostheses and hands; and (b) baseline RT, as a general predictor of processing speed.

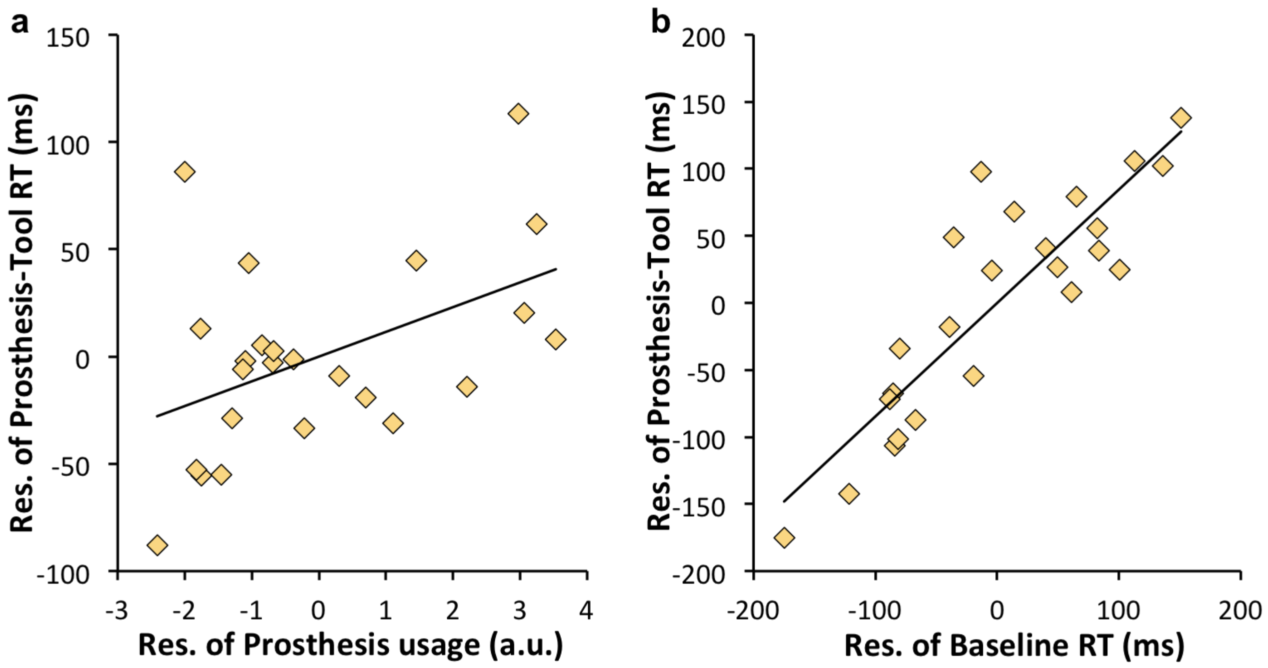

Fig S4. Partial regression plots for the final model of the backwards regression of prosthesis-tool RT. The final model included two predictors: (a) prosthesis usage, suggesting that experience with using a prosthesis leads to a strengthened categorical relationship between prostheses and tools; and (b) baseline RT, as a general predictor of processing speed.

Model	**R**	**R^2_{adj}**	**F**	**p**	**Predictors included**	**β**	**p**
1	0.89	0.73	F(5,18)=13.25	< 0.001	Baseline RT	0.81	**< 0.001**
					Age at limb loss	-0.47	**0.028**
					Years since limb loss	-0.19	0.302
					Prosthesis usage	-0.05	0.713
					Congruency effect	0.04	0.769
2	0.89	0.74	F(4,19)=17.37	< 0.001	Baseline RT	0.80	**< 0.001**
					Age at limb loss	-0.44	**0.016**
					Years since limb loss	-0.17	0.305
					Prosthesis usage	-0.06	0.655
3	0.89	0.75	F(3,20)=24.05	< 0.001	Baseline RT	0.79	< **0.001**
					Age at limb loss	-0.42	**0.015**
					Years since limb loss	-0.18	0.281
4	0.88	0.75	F(2,21)=35.08	< 0.001	Baseline RT	0.78	< **0.001**
					Age at limb loss	-0.29	**0.013**

Table S2: Backwards regression for RT on Prosthesis-Hand trials. Five predictors were entered in the first model and removed with the criteria: probability of F-to-remove ≥ 0.1, probability of F-to-enter ≤ 0.05. Baseline RT, as a measure of general reaction times, was a strong predictor in all models as expected. Additionally, the regression revealed age at limb loss as an experience-based predictor of the conceptual similarity between prostheses and hands.

Model	**R**	**R^2_{adj}**	**F**	**p**	**Predictors included**	**β**	**p**
1	0.91	0.78	F(5,18)=17.16	< 0.001	Baseline RT	0.83	**< 0.001**
					Prosthesis usage	0.19	0.118
					Age at limb loss	-0.27	0.140
					Years since limb loss	-0.14	0.380
					Congruency effect	0.06	0.430
2	0.91	0.79	F(4,19)=22.37	< 0.001	Baseline RT	0.80	**< 0.001**
					Prosthesis usage	0.18	0.122
					Age at limb loss	-0.24	0.139
					Years since limb loss	-0.12	0.424
3	0.91	0.79	F(3,20)=30.10	< 0.001	Baseline RT	0.80	**< 0.001**
					Prosthesis usage	0.17	0.133
					Age at limb loss	-0.15	0.187
4	0.90	0.78	F(2,21)=42.48	< 0.001	Baseline RT	0.83	**< 0.001**
					Prosthesis usage	0.24	**0.026**

Table S3: Backwards regression for RT on Prosthesis-Tool trials. Five predictors were entered in the first model and removed with the criteria: probability of F-to-remove ≥ 0.1, probability of F-to-enter ≤ 0.05. Baseline RT, as a measure of general reaction times, was a strong predictor in all models as expected. Additionally, the regression revealed prosthesis usage as an experience-based predictor of the conceptual similarity between prostheses and tools.

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