A systematic review and meta-analysis of
*Drosophila* short-term-memory genetics: robust reproducibility, but little independent replication

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**Preface**

We invite memory researchers to submit comments, anonymous or signed, by email, social media, or directly to the manuscript. To read text and figures side-by-side on a desktop, please make a duplicate of this file.

**Abstract**

Geneticists have long used olfactory conditioning techniques in *Drosophila* to identify the neurons and genes that mediate learning. While this method has characterized an abundance of memory-related genes, little is known about how these genes induce short-term memory (STM) via signaling pathways; characterizing these networks will be essential to developing mechanistic models of memory formation. Here, we investigated why elucidating the STM pathways has been relatively slow. One possibility is that the STM evidence base is weak due to publication of poorly reproducible results, as has been observed in other fields. We examined this hypothesis by performing a systematic review and subsequent meta-analysis of the STM genetics field. Using several metrics to quantify the variation between discovery articles and follow-up studies, we found that seven genes were highly replicated, showed no publication bias, and had generally high reproducibility. However, the remaining ~80% memory genes have not been replicated since their initial discovery. Although we observed only a few studies that investigated gene interactions, the reviewed genes could together account for >1000% memory. This large summed effect size indicates either that some of the gene findings are not reproducible, that many memory genes participate in shared pathways, or that current protocols lack the specificity needed to identify core plasticity memory genes. Mechanistic theories of memory and cognition will require the convergence of evidence from system, circuit, cellular, molecular, and genetic experiments. As this study demonstrates, systematic data synthesis is an essential tool for this integrated brain science.
Introduction

Learning is the process by which external sensory experiences and internal states lead to behavioral adaptation. Learning manifests as physiological changes in the brain, the most prominent of which are alterations to the connections between neurons, known as synaptic plasticity (Takeuchi et al., 2014). The plasticity theory of learning draws on findings from numerous experimental systems, including vertebrate models, most notably mouse, and invertebrate models, such as the sea slug *Aplysia californica* and the vinegar fly *Drosophila melanogaster* (Bailey et al., 2015; Cognigni et al., 2017; Ehmann et al., 2017). For decades, the *Drosophila* model has been used to characterize the neurogenetic mechanisms underlying memory formation (Davis, 2011). Much of our knowledge of the neurogenetics of learning derives from experiments using Pavlovian odor (olfactory) conditioning (Quinn et al., 1974). Olfactory conditioning uses the simultaneous presentation of an odor paired with an inherently valued stimulus, usually painful electric shocks or a nutritious sugar meal (Tempel et al., 1983; Tully and Quinn, 1985). Conditioned animals display altered approach/avoidance responses to subsequent odor presentation. When the post-learning trial is performed within minutes of conditioning, the response is referred to as short-term memory (STM). Starting with *rutabaga* (*rut*) and *dunce* (*dnc*) in the 1980s, such experiments performed on *Drosophila* mutants have identified a number of STM genes (Heisenberg, 2003; Keene and Waddell, 2007; Tomchik and Davis, 2013).

Many STM genes are predominantly expressed in a single brain structure, but an integrated model of the overall STM signaling architecture is lacking. This absence contrasts starkly with other *Drosophila* gene systems. For example, the *Drosophila* signaling networks underlying both embryonic development (Perrimon et al., 2012; St Johnston and Nüsslein-Volhard, 1992) and the circadian clock (Hardin, 2011) have been characterized in detail. In those cases, our molecular-genetic knowledge has reached such an extent that it informs mathematical models that can recreate key system properties (Fathallah-Shaykh et al., 2009; Segal et al., 2008). It is unknown why genetics has succeeded in defining those aforementioned systems, while delineating similarly complete plasticity pathways has been hard.

Many hypotheses could be put forward to explain why the genetics of memory formation have not yet reached a level of clarity. One such possibility is a weak evidence base. For instance, circadian-rhythm genetics benefits from very large effect-sizes, while memory phenotypes are smaller (Takahashi et al., 2008), making reliable measurements more difficult. Many scientists believe that there is a reproducibility crisis in biomedical
research (Baker, 2016), and intense investigations into data reproducibility have ensued (eLife, 2017; Lithgow et al., 2017), including in the fields of cancer research (Begley and Ellis, 2012), drug target identification (Prinz et al., 2011), and human psychology (Anderson et al., 2016; Gilbert et al., 2016; Open Science Collaboration, 2015a). In medical research, scientists routinely assess a field’s evidence base with statistical synthesis (Haidich, 2010). Such meta-research—although relatively rare in the basic biomedical sciences—is growing in importance (Claridge-Chang and Assam, 2016; Nature methods, 2016; Yildizoglu et al., 2015).

To test the hypothesis that the development of an integrated model of STM has been frustrated by irreproducibility, we evaluated the evidence base with a synthetic analysis of loss-of-function alleles. Our systematic review identified 32 STM genes, of which 23 were amenable to meta-analysis. We applied three metrics across several types of replication to quantify reproducibility. The findings on replicated genes were consistent, and statistical evidence for publication bias was absent. These findings refute our hypothesis and confirm good reproducibility. However, independent replication was rare: most replication was reported in follow-up studies from the discovery group; and only seven of the 32 genes—just 22%—were replicated independently. These results indicate that the Drosophila memory-genetics evidence base is divided: a low replication rate for most genes, with robust data integrity for a selected few.
Materials and Methods

Information sources and database search
This review was conducted by searching PubMed and Embase databases on 8th April 2017 with the phrase: “Drosophila AND (learning OR memory) AND (olfactory OR olfaction OR T-maze OR "T maze" OR odorant OR odor) NOT review[Publication Type]”. The query returned 648 and 559 publications from Pubmed and Embase, respectively. The results were downloaded as .csv files, and the two sources were merged. Publications that were not research articles or not written in English were excluded; this resulted in the identification of 743 articles (Figure 1).

Eligibility criteria
The systematic review included studies that met the following six criteria: (1) report STM performance defined as ≤ 5 min memory (Heisenberg, 2003; Yildizoglu et al., 2015); (2) measure STM in adult Drosophila melanogaster; (3) focus on homozygous loss-of-function mutations, including broad RNA interference (RNAi) knockdown mutants; (4) use the T-maze assay; (5) report the full performance index (PI) score for control and experimental flies; and (6) use mutants with intact locomotor activity, olfactory acuity, and brain development processes. Due to the rapid transition of STM to middle-term memory (Heisenberg, 2003), we excluded experiments where the time interval between training and testing was >5 min.

Study selection
Articles were screened for exclusion in four successive stages: (1) title—375 excluded, (2) abstract—232 excluded, (3) full text—84 excluded, and (4) experimental design—2 excluded. After screening, 50 articles that fully satisfied our selection criteria were taken forward for data extraction and meta-analyses (Figure 1).
Figure 1. Systematic review procedure

PubMed and Embase searches performed in April 2017 identified 743 articles relevant to *Drosophila* short-term memory genes. A four-stage screening process excluded 693 articles, leaving 50 for further review and meta-analyses.
**Data extraction**

We extracted the following experimental parameters from the 50 included articles: author, year, figure and panel number, experimental genotypes, control genotypes, full PIs of all conditions, standard error of the mean (SEM), sample size (N), temperature, relative humidity, odor pairs, shock voltage, current type, number of training, number of shocks per training, training duration, and the training–testing time interval. If tabulated data were not available, the PI and SEM were extracted from graphs using the Adobe Illustrator (Adobe Systems USA) measurement tool and extrapolated from the y-axis length to obtain a numerical value.

**Summary measures**

The PI of the control-group STM can vary considerably between studies (Yildizoglu et al., 2015), which renders inter-study comparisons challenging. As such, we used performance percent change (PPC) as previously described (Yildizoglu et al., 2015). For each experiment, the PPC was calculated as follows:

\[
PPC = \frac{P_{\text{exp}} - P_{\text{ctrl}}}{P_{\text{ctrl}}} \times 100
\]

The standard error (SE) of the PPC was calculated using the delta approximation (Cramer, 1946; Oehlert, 1992; Yildizoglu et al., 2015), as follows:

\[
SE_{\text{pooled}} = \frac{\text{mean experimental}}{\text{mean control}} \sqrt{\left( SE_{\text{experimental}} / \text{mean experimental} \right)^2 + \left( SE_{\text{control}} / \text{mean control} \right)^2}
\]

**Outlier exclusion**

Outliers were excluded from the dataset based on Z-score values, following best practice (Altman, 1968; Pagano et al., 2000). Effect sizes with a Z-score >2 were removed from the dataset. In total, eight experiments conducted on five alleles were excluded and are greyed-out in the corresponding forest plots.

**Meta-analysis calculations**

Where multiple experiments were available (for 23/32 genes), data were meta-analyzed with a random effects model (RE model) to calculate summary effect sizes. Subgroup analyses were also performed on the genes with data for multiple alleles. (When a subgroup had only one experiment, the fixed effects model was used by default.) Both analyses used the metafor package in R (Viechtbauer and Others, 2010).

**Publication bias**

For genes where ≥10 internal replicates were available, publication bias was assessed by constructing a funnel plot of effect sizes (Sterne et al.,...
Effect sizes and corresponding precisions were plotted against each other and inspected for symmetrical distribution around the meta-analytic mean (Light and Pillemer, 1984; Liu, 2011). Egger’s method was then applied to test for bias in each gene’s data (Egger et al., 1997).

**Replication terminology**

An *ad hoc* literature search failed to identify definitions of ‘replication’ or ‘reproducibility’ that are broadly accepted and/or statistically formalized. In the context of genetic experiments for STM, we found that *replication* could have at least four meanings: (1) the sample size of a single experiment; (2) multiple experiments reported in a single discovery study; (3) replication reported in a follow-up study by the discovery laboratory; and (4) genuinely independent replication conducted by at least one group of scientists not involved in the original study. By this taxonomy, definitions 1, 2, and 3 represent *dependent replication* and definition 4 represents *independent replication*, which is the most valuable. In the context of loss-of-function genetics, there is the possibility of quantitative and qualitative differences between various alleles; we refer to experiments conducted with an identical allelic state as *allelic replication*. There is also a delineation between efforts to exactly recreate all conditions of the discovery experiment—*direct replication*—and experiments that vary conditions, with the ability to generalize discovery findings—*conceptual replication* (Ioannidis, 2012; Makel et al., 2012).

**Reproducibility measures**

Published definitions of *reproducibility* vary widely (McNaught and Wilkinson, 1997; Open Science Collaboration, 2015a; Patil et al., 2016). For the purpose of this meta-analysis, we defined reproducibility as the quantifiable extent of agreement between replicate effect sizes (Open Science Collaboration, 2015b). To estimate this, we adopted three statistical methods. Firstly, we employed heterogeneity ($I^2$), which measures the proportion of variance that cannot be attributed to sampling error. This measure is widely used in meta-analyses and is precisely defined (Higgins et al., 2003); close agreement between studies produces a favorably low heterogeneity. However, poor precision in the constituent studies of a meta-analysis can also result in low heterogeneity, giving the false impression of good reproducibility. To address this limitation, we also used the mean absolute difference (MAD) between all replicates in a set (see below). Although MAD does not incorporate meta-analytic weighting, it has the benefit of being an intuitive, direct measure of overall discrepancies between means and is not confounded by imprecision. Finally, we generated violin plots to compare discovery effect sizes with replicate effect sizes (Open Science Collaboration, 2015b).
Heterogeneity assessment of reproducibility

Meta-analysts source data from several studies to estimate the summary effect size of an intervention. This procedure also inspects the assumption that the included effect sizes are drawn from a common population. Heterogeneity ($I^2$) describes the proportion of meta-analytic variance that is attributable to samples being drawn from different populations, and is calculated using the following formula:

$$I^2 = 100\% \times \frac{(Q - df)}{Q}$$

where $Q$ is Cochran's heterogeneity statistic and $df$ is the degrees of freedom (Higgins et al., 2003).

An $I^2 < 50\%$ is considered low, an $I^2$ between 50–75% is considered moderate, and an $I^2 > 75\%$ is considered high heterogeneity (Higgins et al., 2003). Here, we calculated $I^2$ for three groupings, namely: 1) all variants of a gene; 2) inter-allelic heterogeneity between the allelic subgroups; and 3) intra-allelic heterogeneity. We used the metafor library in R (Viechtbauer and Others, 2010).

MAD assessment of reproducibility

Discrepancies between all replicates in the units of the effect size can be reported as MAD (also known as Gini’s mean difference) between all effect sizes in a meta-analysis (David, 1968). We calculated MAD as follows:

$$MAD = \frac{\sum_{i=1}^{k} \sum_{j=1}^{i} |PPC_i - PPC_j|}{\binom{i}{2}}$$

where PPC is the performance percent change, and $k$ is the total number of PPCs.

A histogram, QQ-plot, and Shapiro-Wilk test showed that the distribution of effect sizes from the largest meta-analysis (rut, Figure 4) were not normally distributed (Shapiro-Wilk $P = 0.0015$; plots not shown). As MAD is preferred over standard deviation when the data are not normally distributed (Yitzhaki, 2002), we adopted the MAD assessment for all gene analyses. As for $I^2$, MAD compared: intra-allelic replicates; interallelic replicates; and all loss-of-function data for a given gene. Unlike $I^2$, there are no pre-established guidelines that relate arbitrary metric thresholds to verbal descriptors. To place MAD values in context with PPC effect sizes, we chose $\geq 20\%$ as an arbitrary threshold of high MAD, because only 3/23 meta-analyzed genes had effect sizes $\leq |20\%|$ (Figure 3).
Results

Experimental conditions vary across studies

We found extensive methodological differences between the 50 published studies (Figure 2). The most commonly used odor pair of 3-octanol, 4-methylcyclohexanol (OCT/MCH) (Tully and Quinn, 1985) was used to condition flies in <50% of conditioning experiments, while much of the remainder used benzaldehyde combinations. The experimental temperature and humidity settings also varied widely. Most protocols delivered 12 foot shocks (at either 60 V or 90 V); most training periods lasted for 60 s; the shock type (AC or DC) was not reported for half of the protocols (143 of the 278).

Figure 2. Methodological variation across conditioning experiments

Distribution of experimental conditions across the 278 analysed experiments from 50 articles. EB = ethyl butyrate, EA = ethyl acetate, AA = amyl acetate, IAA = isoamyl acetate, BA = benzaldehyde, NA = not available.
Most STM genetic studies have not been independently replicated

The reviewed articles implicate 32 genes in *Drosophila* STM; we summarized the meta-analytic results for each gene in Figure 3. Out of the 32 genes, findings on 17 were replicated in at least one follow-up study. However, 10 of these were internal replications conducted by the discovery group; independent replication studies have been conducted for just seven genes. Moreover, only two genes were characterized by >2 independent replicate studies: *rut* (6 replicates) and *dnc* (3 replicates). The other five genes (*Neurofibromin 1 (NF1), rugose (rg), Dopamine transporter (DAT), dopamine 1-like receptor (Dop1R1) and fragile X mental retardation 1 (Fmr1)) were replicated by a single independent study. Detailed forest plots and subgroup analyses for individual genes and alleles are shown in Supplementary Figures S1–S15.
Figure 3. Meta-analytic findings for 32 STM-relevant genes

Summary effect sizes were calculated as performance percent change (PPC) relative to controls (see Methods). Effect sizes reported by independent replications, non-independent replications, and single studies are represented as gold, silver, and bronze diamonds, respectively. Effect sizes reported by one experiment are represented as black dots. Memory alterations in *Drosophila* mutants ranged from +20% (an STM improvement in *trbl* mutants), to −134% (an STM defect in *ple* mutants). The studies show a wide range of iterations, from 6–153. Each iteration reports a full PI score that is derived from ~100 conditioned flies. The total number of experiments for each gene ranges from 1 to 59, where *5HT1B, 5HT2, 5HT7, ple, elm, nemy* and *Syn* are represented by only one experiment and *rut* is represented by 59 experiments. Abbreviations are as follows: *ple* = *pale*; *Dop1R1* = Dopamine 1-like receptor 1; *5HT1B* = 5-hydroxytryptamine receptor 1B; *dnc* = dunce; *rut* =

| Gene   | Iterations (N) | Experiments | Studies | Independent replicates | PPC [95% CI] |
|--------|----------------|-------------|---------|------------------------|-------------|
| ple    | 9              | 1           | 1       | 0                      | −134 [−150, −56] |
| *Dop1R1* | 88             | 14          | 2       | 1                      | −96 [−100, −92] |
| *5HT1B* | 8              | 1           | 1       | 0                      | −75 [−90, −60] |
| *dnc*  | 176            | 23          | 6       | 3                      | −67 [−72, −63] |
| *rut*  | 513            | 59          | 14      | 6                      | −57 [−61, −53] |
| *Fmr1* | 18             | 2           | 2       | 1                      | −53 [−98, −9] |
| *5HT2A* | 8              | 1           | 1       | 0                      | −52 [−67, −37] |
| *gish* | 8              | 1           | 1       | 0                      | −52 [−67, −36] |
| *sra*  | 40             | 8           | 1       | 0                      | −51 [−57, −45] |
| *srb*  | 150            | 23          | 2       | 0                      | −49 [−58, −39] |
| *elm*  | 6              | 1           | 1       | 0                      | −44 [−71, −17] |
| *rg*   | 108            | 14          | 2       | 1                      | −43 [−53, −32] |
| *Dop2R* | 135            | 15          | 1       | 0                      | −41 [−47, −35] |
| *Drep2* | 130            | 9           | 1       | 0                      | −40 [−47, −32] |
| *5HT7* | 8              | 1           | 1       | 0                      | −38 [−52, −24] |
| *S6KII* | 39             | 3           | 1       | 0                      | −37 [−52, −23] |
| *Pp1–87B* | 15          | 3           | 1       | 0                      | −30 [−50, −10] |
| *amn*  | 28             | 5           | 3       | 0                      | −30 [−49, −11] |
| *NF1*  | 160            | 26          | 2       | 1                      | −28 [−34, −22] |
| *mbm*  | 12             | 2           | 1       | 0                      | −28 [−53, −2] |
| *Nmdar1* | 16            | 2           | 1       | 0                      | −28 [−40, −15] |
| *DAT*  | 29             | 4           | 2       | 1                      | −27 [−36, −19] |
| *Syn*  | 9              | 1           | 1       | 0                      | −26 [−44, −8] |
| *14–3–3ζ* | 203          | 24          | 2       | 0                      | −26 [−29, −23] |
| *Fas2* | 110            | 19          | 1       | 0                      | −26 [−32, −19] |
| *Pkαι–R1* | 18           | 3           | 1       | 0                      | −25 [−32, −17] |
| *Adf1* | 54             | 6           | 1       | 0                      | −21 [−27, −15] |
| *PQBP1* | 18             | 2           | 1       | 0                      | −20 [−31, −9] |
| *nemy* | 8              | 1           | 1       | 0                      | −17 [−22, −13] |
| *Dop1R2* | 8              | 1           | 1       | 0                      | −15 [−36, 7] |
| *aru*  | 76             | 4           | 1       | 0                      | −14 [−31, 2] |
| *trbl* | 12             | 2           | 1       | 0                      | 20 [6, 33] |
rutabaga; Fmr1 = Fragile X mental retardation 1; 5HT2A = 5-hydroxytryptamine receptor 2A; gish =
gilgamesh; sra = sarah; scb = scab; elm = ethanol sensitive with low memory; rg = rugose; Dop2R =
Dopamine 2-like receptor; drep-2 = DNA fragmentation factor-related protein 2; 5HT7 = 5-
hydroxytryptamine receptor 7; S6KII = Ribosomal protein S6 kinase III; Pp1-87B = Protein
phosphatase 1 at 87B; amn = amnesiac; NF1 = Neurofibromin 1; mbm = mushroom body miniature;
Nmdar1 = NMDR receptor; DAT = Dopamine transporter; Syn = Synapsin; 14-3-3-Zeta = 14-3-3-Zeta;
Fas2 = Fasciclin 2; PKA-RI = Protein kinase, cAMP-dependent, regulatory subunit type 1; Adf1 = Adh
transcription factor 1; PQBP1 = Poly-glutamine tract binding protein 1; nemy = no extended memory;
Dop1R2 = Dopamine 1-like receptor 2; aru = arouser; trbl = tribbles.
**Rut function determines ~57% of STM**

*Rut* was one of the first genes implicated in STM (Livingstone et al., 1984): it encodes an adenylyl cyclase that generates cyclic adenosine monophosphate (cAMP) (Levin et al., 1992). Extending an earlier meta-analysis (Yildizoglu et al., 2015), our review identified *rut* experiments on 12 loss-of-function alleles and heteroallelic combinations. The STM phenotypes of two alleles (*rut*2080 and *rut*t) have been studied by several groups, but other alleles have not been replicated independently. Two of the *rut* loss-of-function alleles (*rut*769 and *rut*1951) showed a much smaller STM impairment compared to the others (Figure 4). These changes in effect size may be due to different degrees of *rut* deficiency. The aggregate STM reduction caused by loss-of-function *rut* mutations is ~57% [95CI –61, –53] with a moderate overall heterogeneity ($I^2$) of 65%.
| Study       | Figure | Allele       | %PPC [95CI] |
|------------|--------|--------------|-------------|
| Beck 2000  | 7      | rut<sup>1068</sup> | ▲ -41 [−100, 31] |
| Blum 2009  | 1A     | rut<sup>1068</sup> | −69 [−85, −52] |
| Buchanan 2010 | 1B  | rut<sup>1068</sup> | −32 [−83, −25] |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −50 [−90, −6]  |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −40 [−80, 1]   |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −40 [−56, −23] |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −30 [−49, −10] |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −20 [−53, 12]  |
| Han 1992   | 2A     | rut<sup>1068</sup> | −70 [−100, −40] |
| McGuire 2003 | 2A  | rut<sup>1068</sup> | −59 [−71, −48] |
| Zars 2000  | 1      | rut<sup>1068</sup> | −43 [−71, −15] |
| McGuire 2003 | 2A  | rut<sup>1068</sup> | −37 [−65, −9]  |
| McGuire 2003 | 2A  | rut<sup>1068</sup> | −60 [−75, −44] |
| McGuire 2003 | 2A  | rut<sup>1068</sup> | −61 [−75, −47] |
| Akaal 2006  | 3A     | rut<sup>1068</sup> | −66 [−86, −45] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −66 [−88, −45] |
| Akaal 2006  | 3C     | rut<sup>1068</sup> | −72 [−82, −61] |
| Akaal 2006  | 3A     | rut<sup>1068</sup> | −49 [−81, −17] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −73 [−100, −45] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −71 [−89, −53] |
| Akaal 2006  | 3C     | rut<sup>1068</sup> | −78 [−100, −45] |
| Akaal 2006  | 3A     | rut<sup>1068</sup> | −49 [−69, −30] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −64 [−84, −43] |
| Akaal 2006  | 3C     | rut<sup>1068</sup> | −59 [−72, −46] |
| Akaal 2006  | 3A     | rut<sup>1068</sup> | −77 [−91, −64] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −71 [−82, −59] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −74 [−98, −49] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −76 [−91, −61] |
| Akaal 2006  | 3A     | rut<sup>1068</sup> | −71 [−84, −58] |
| Akaal 2006  | 3B     | rut<sup>1068</sup> | −64 [−82, −46] |
| Mao 2003   | 3A     | rut<sup>1068</sup> | −48 [−78, −18] |
| Mao 2003   | 3A     | rut<sup>1068</sup> | −71 [−97, 1] |
| Beck 2000  | 7      | rut<sup>1068</sup> | −18 [−75, 38] |
| Beck 2000  | 7      | rut<sup>1068</sup> | −18 [−52, 16] |
| Buchanan 2010 | 2A  | rut<sup>1068</sup> | −10 [−37, 18] |

**RE Model**

**Heterogeneity: F = 66%**

Test for heterogeneity: Q(df=31) = 124.7 (P < 0.0001)

| Study       | Figure | Allele       | %PPC [95CI] |
|------------|--------|--------------|-------------|
| Blum 2009  | 1A     | rut<sup>1068</sup> | −62 [−86, −38] |
| Cressy 2014 | 3A    | rut<sup>1068</sup> | −51 [−65, −37] |
| Cressy 2014 | 3B    | rut<sup>1068</sup> | −54 [−65, −42] |
| Cressy 2014 | 3D    | rut<sup>1068</sup> | −60 [−80, −39] |
| Gass 2000  | 2A     | rut<sup>1068</sup> | −46 [−65, −27] |
| Han 1992   | 2A     | rut<sup>1068</sup> | −67 [−90, −44] |
| Han 1992   | 2B     | rut<sup>1068</sup> | −75 [−92, −58] |
| Scheunemann 2012 | 1D | rut<sup>1068</sup> | −59 [−85, −32] |
| Scheunemann 2012 | 5A | rut<sup>1068</sup> | −70 [−89, −51] |
| Scheunemann 2013 | 1E/F  | rut<sup>1068</sup> | −37 [−75, 0] |
| Scheunemann 2013 | 1E/F  | rut<sup>1068</sup> | −67 [−86, −45] |
| Scheunemann 2013 | 1E/F  | rut<sup>1068</sup> | −18 [−79, 53] |

**RE Model**

**Heterogeneity: F = 19%**

Test for heterogeneity: Q(df=10) = 11.3667 (P = 0.3297)

| Study       | Figure | Allele       | %PPC [95CI] |
|------------|--------|--------------|-------------|
| Han 1992   | 2A     | rut<sup>1068</sup> | −57 [−80, −34] |
| Han 1992   | 2B     | rut<sup>1068</sup> | −58 [−85, −30] |

**RE Model**

**Heterogeneity: F = 0%**

Test for heterogeneity: Q(df=1) = 0.0007 (P = 0.9788)

| Study       | Figure | Allele       | %PPC [95CI] |
|------------|--------|--------------|-------------|
| Han 1992   | 2A     | rut<sup>1068</sup> | −54 [−81, −27] |
| Han 1992   | 2B     | rut<sup>1068</sup> | −56 [−86, −25] |

**RE Model**

**Heterogeneity: F = 0%**

Test for heterogeneity: Q(df=1) = 0.0063 (P = 0.9368)

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**Figure 4, Part 1 of 2.**
**Figure 4. Loss of *rut* function across different alleles reduces STM by 57%**

Meta-analysis of data from *rut* loss-of-function experiments reveals an overall STM reduction of -57% [95CI -61, -53] with an $I^2$ of 65%. Black squares represent the mean performance percent change (PPC) for corresponding experiments; square sizes represent the relative contribution of each experiment to the meta-analytic average. Data sources are indicated in the study and figure columns; alleles are indicated in their own column. Blue diamonds represent the effect size of the allelic subgroups and the red diamond indicates the overall effect size for *rut*. All error bars (including diamond vertices) represent the 95% CI. The grey-coloured rows indicate the outliers.
that were excluded from the calculations based on a Z-score outlier filter (see Methods). This data presentation format is repeated for all other forest plots. FE = fixed effects; RE = random effects.

**Dnc lesions reduce STM by two-thirds**

*Dnc* was the first *Drosophila* gene discovered to modulate memory (Dudai et al., 1976). Subsequent studies have found that *dnc* encodes a cAMP-specific phosphodiesterase that likely consumes the cAMP generated by RUT activity (Davis and Kiger, 1981). Here, we identified experiments on seven *dnc* alleles and heteroallelic combinations. The *dnc* meta-analysis revealed a summary effect size of -67% [95CI -72, -63], with moderate heterogeneity, $I^2 = 61\%$ (Figure 5).
Figure 5. Loss of dnc function across different alleles reduces STM by 67%.

Meta-analysis of dnc loss-of-function data indicates an overall effect size of -67% [95CI -72, -63] with an $I^2$ of 61%. FE = fixed effects; RE = random effects.
**Nf1 function determines ~33% STM**

*Nf1* encodes a Ras-specific GTPase activating protein; in humans, mice and flies, *Nf1* lesions elicit various effects including memory deficits (Guo et al., 2000). *Drosophila* NF1 interacts with RUT (Guo et al., 2000) in the mushroom body (MB) (Buchanan and Davis, 2010). Here, we identified two STM studies on three loss-of-function *Nf1* alleles: studies on two alleles (*Nf1*^P1^ and *Nf1*^P2^) have been independently replicated but the effect of *Nf1*c00617 has been investigated only once. Our meta-analysis of all three alleles showed an overall STM decrease of ~28% [95CI –34, –22] (Figure 6). However, compared to *Nf1*^P1^ and *Nf1*^P2^ alleles (~31% and ~36% respectively), the effect size of *Nf1*c00617 (~12%) was low. Because the reduction in male body size associated with *NF1* deficiency was also relatively mild in *Nf1*c00617 mutants, (Buchanan and Davis, 2010), these findings suggest that *Nf1*c00617 is a weak hypomorph. As such, the best estimate for the contribution of *Nf1* to STM is about one-third.
**Figure 6.** Loss of \( Nf1 \) function across different alleles reduces short-term memory by \(-28\%\)

Meta-analysis of \( Nf1 \) loss-of-function mutants produced an overall effect size of \(-28\% \) [95CI -34, -22] with an \( I^2 \) of 63%. RE = random effects.
Loss of rg function affects STM

Rg encodes an A-kinase anchoring protein (AKAP) that is involved in nervous system development (Shamloula et al., 2002). Loss of rg function decreases STM, while leaving other types of memory intact (Volders et al., 2012). We identified STM studies on six allelic states: three rg hypomorphs (rg1, rgKG02343, rgγ5), an RNAi rg knockdown, a heteroallelic mutant (rg1/rgKG02343) and one amorph (rgFDD). The overall decrease in STM was ~43% (Figure 7), but only findings on the rg1 allele were independently replicated (Volders et al., 2012; Zhao et al., 2013). Inducing aberrant MB morphology (Volders et al., 2012; Zhao et al., 2013), the rg null has the strongest effect of all rg alleles on STM (~89%).
| Study       | Figure | Allele                      | %PPC [95CI] |
|-------------|--------|-----------------------------|-------------|
| Volders 2012| 2A     | $r_g^s$                      | −35 [−58, −11] |
| Zhao 2013   | 3A     | $r_g^s$                      | −33 [−57, −10] |
| Zhao 2013   | 3B     | $r_g^s$                      | −24 [−43, −4]  |
| Zhao 2013   | 1C     | $r_g^s$                      | −28 [−47, −10] |
| Volders 2012| 2C     | $r_g^s;UAS−r_g+cDNA$        | −28 [−50, −5]  |
| RE Model    |        |                             | −29 [−39, −19] |
| Volders 2012| 2A     | $r_g^s$                      | −45 [−65, −24] |
| Volders 2012| 2F     | $r_g^{FDD}$;UAS−mNBEA+cDNA | −52 [−79, −24] |
| Volders 2012| 3F     | $r_g^{FDD}$;UAS−r_g+cDNA    | −38 [−66, −10] |
| Volders 2012| 2F     | $r_g^{FDD};UAS−mNBEA+cDNA$  | −42 [−73, −10] |
| Volders 2012| 3F     | $r_g^{FDD};UAS−r_g+cDNA$    | −33 [−66, 0]   |
| Volders 2012| 2C     | $r_g^{FDD};UAS−r_g+cDNA$    | −48 [−79, −16] |
| RE Model    |        |                             | −43 [−55, −32] |
| Zhao 2013   | 1C     | $r_g^{K02343}$               | −32 [−59, −4]  |
| FE Model    |        |                             | −32 [−59, −4]  |
| Zhao 2013   | 1D     | $r_g^i/r_g^{K02343}$         | −37 [−51, −22] |
| FE Model    |        |                             | −37 [−51, −22] |
| Zhao 2013   | 4B     | elav−Gal4;UAS−r_gRNAi;Gal80ts| −37 [−58, −16] |
| FE Model    |        |                             | −37 [−58, −16] |

**Figure 7. Loss of $r_g$ function across different alleles leads to an STM reduction of 43%**

Meta-analysis of $r_g$ produced an overall effect size of −43% [95CI -53, -32]. Compared to the other alleles, the effect size of the amorphic $r_g^{FDD}$ was more than twice as high: −89%. Heterogeneity across experiments was moderate $I^2 = 72\%$. FE = fixed effects; PPC = performance percent change; RE = random effects.
Loss of *Fmr1* elicits a relatively mild STM phenotype

*Fmr1* encodes the fly ortholog of the human FMRP RNA-binding protein associated with Fragile X mental retardation (Zhang et al., 2001). Patients with Fragile X syndrome contain a GGG-triplet expansion in the 5′ untranslated region of *Fmr1*, which causes hypermethylation and transcriptional silencing (Coffee et al., 2012; Verkerk et al., 1991). Our review identified two studies relating *Fmr1* function to STM: one probed the effect of a loss-of-function allele *Fmr1*Δ50M, while the other used RNAi to knock down *Fmr1* (Coffee et al., 2012; Kanellopoulos et al., 2012). The *Fmr1*Δ50 allele in the homozygous state decreased STM by −77%, while pan-neuronal RNAi knockdown reduced STM by −31% (Figure 8).
| Study               | Figure | Allele                   | %PPC[95CI]             |
|--------------------|--------|--------------------------|------------------------|
| Kanellopoulos 2012 | 2B     | elav-Gal4; Gal80; UAS–Fmr1--RNAi | -31 [-54, -8]         |
| Coffee 2011        | 7B     | Fmr1^{50M}               | -77 [-100, -50]        |

**Figure 8. Loss of Fmr1 across different alleles reduces STM by 53%**

Meta-analysis of Fmr1 indicates an effect size of -53% [95CI -98, -9]. RE = random effects.
Four dopamine-signalling genes affect STM

Dopamine is instrumental to a wide range of behaviours, including STM, and >12 Drosophila genes are associated with dopamine metabolism or signaling (Yamamoto and Seto, 2014). Of these 12 dopamine-related genes, the systematic review identified STM studies performed on four: DAT, ple, Dop1R1, and Dop2R, discussed below.

DAT. Dopamine shuttles across the plasma membrane via the transporter, DAT (Neckameyer and White, 1993; Riemensperger et al., 2011; Zhang et al., 2008). An imbalance in dopamine levels due to altered DAT function is associated with various neurological disorders and addiction in humans (Ueno, 2003; Yang et al., 2007). We found two studies (Ueno et al., 2012; Zhang et al., 2008) that showed that loss of DAT function decreases STM by about –27% (Figure 9A).

Ple. The Drosophila pale locus encodes the biosynthetic enzyme tyrosine hydroxylase, which is critical for dopamine production. While systemic knockout of ple is lethal, one allele abolishes ple function exclusively in the central nervous system (CNS), thus permitting viability (Riemensperger et al., 2011). Strikingly, absence of dopamine in the CNS inverts STM polarity: instead of avoiding the shock-associated odors, ple mutants actively seek them out—exhibiting a –134% reduction in STM (Figure 3).

Dop1R1 and Dop2R. Four different dopamine receptors have been described in flies: Dop1R1, Dop1R2, Dop2R, and DopEcR (Feng et al., 1996; Gotzes et al., 1994; Hearn et al., 2002; Srivastava et al., 2005). As in mammals, the Drosophila D1-type receptors Dop1R1 and Dop1R2 increase cAMP levels upon dopamine agonism (Beaulieu et al., 2015; Boto et al., 2014). Dop1R1 is expressed in the fan-shaped body, the ellipsoid body and the MB; this receptor has important roles in sleep, arousal, and memory (Andretic et al., 2005; Lebestky et al., 2009; Ueno et al., 2012). In the two studies on this gene (Kim et al., 2007; Qin et al., 2012), two Dop1R1 loss-of-function alleles (Dop1R1<sup>In(3LR)234</sup> and Dop1R1<sup>f02676</sup>) almost completely eliminated learning, with an average STM reduction of –96% (Figure 9B). Dop2R is highly expressed in the MB and decreases cAMP levels in response to dopamine agonism (Scholz-Kornehl and Schwärzel, 2016). We identified one STM study describing a hypomorphic Dop2R mutation that reduced STM by –41% (Scholz-Kornehl and Schwärzel, 2016) (Figure 9C).
### A

| Study       | Figure | Allele               | %PPC [95CI]  |
|-------------|--------|----------------------|--------------|
| Zhang 2008  | 2A     | DAT<sup>hox</sup>     | -22 [-36, -7]|
| Zhang 2008  | 2C     | DAT<sup>hox</sup>-TH-Gal4 | -26 [-43, -10] |
| Zhang 2008  | 2C     | DAT<sup>hox</sup>–UAS–dDAT | -30 [-45, -14] |
| Ueno 2014   | 6A     | DAT<sup>hox</sup>–UAS–dDAT | -49 [-79, -18] |

**RE Model**

Heterogeneity: Inter-allelic F = Not applicable

Overall F = 0%

Test for heterogeneity: Q(df=5) = 15.895 (P = 0.009)

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### B

| Study       | Figure | Allele               | %PPC [95CI]  |
|-------------|--------|----------------------|--------------|
| Kim 2007    | 2C     | Dop1R<sup>1K0776</sup> | -101 [-100, -66] |
| Kim 2007    | 4C     | Dop1R<sup>1K0776</sup> | -93 [-100, -50] |
| Qin 2012    | 2B     | Dop1R<sup>1K0776</sup> | -104 [-100, -92] |
| Qin 2012    | 2C     | Dop1R<sup>1K0776</sup>–UAS–DopR | -102 [-100, -79] |
| Qin 2012    | 2D     | Dop1R<sup>1K0776</sup> | -96 [-100, -84] |
| Qin 2012    | 4A     | Dop1R<sup>1K0776</sup> | -94 [-100, -83] |
| Qin 2012    | 4B     | Dop1R<sup>1K0776</sup> | -102 [-100, -86] |
| Qin 2012    | 2A     | Dop1R<sup>1K0776</sup>–UAS–DopR | -92 [-100, -81] |
| Qin 2012    | 2A     | Dop1R<sup>1K0776</sup>–NP1131–Gal4 | -103 [-100, -71] |
| Qin 2012    | 2A     | Dop1R<sup>1K0776</sup>–NP3061–Gal4 | -96 [-100, -73] |
| Kim 2007    | 4B     | Dop1R<sup>1K0776</sup> | -79 [-98, -60] |

**RE Model**

Heterogeneity: F = 0%

Test for heterogeneity: Q(df=3) = 3.0138 (P = 0.9637)

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### Figure 9, Part 1 of 2.
Figure 9. Meta-analyses of genes associated with dopamine signalling

A. Meta-analysis of DAT indicates an overall effect size of \(-27\%\) [95CI -36,-19].

B. Meta-analysis of Dop1R1 indicates an effect size of \(-96\%\) [95CI -100,-92]. \(Dop1R1^{In(3LR)234}\) and \(Dop1R1^{f02676}\) are also known as \(Dop1R1^{dumb1}\) and \(Dop1R1^{dumb2}\), respectively.

C. Disruption of Dop2R has an overall effect size of \(-41\%[95CI -47,-35]\) on short-term memory. PPC = performance percent change; RE = random effects.
Phosphorylation factors are key to STM

Current hypotheses for the mechanisms underlying memory formation invoke protein phosphorylation and dephosphorylation (Alberini, 2009; Kandel, 2012; Margulies et al., 2005). Five phosphorylation-related genes are associated with STM: Protein kinase, cAMP-dependent, regulatory subunit type 1 (Pka-R1) (Goodwin et al., 1997); Ribosomal S6 serine/threonine kinase (S6KII) (Putz et al., 2004); Protein phosphatase 1 at 87B (Pp1-87B) (Asztalos et al., 1993); Sarah (sra) (Chang et al., 2003); and gilgamesh (gish) (Tan et al., 2010). Of these, Pka-R1 and S6KII encode kinases (Kalderon and Rubin, 1988; Kim et al., 2006), Pp1-87B encodes a phosphatase (Baksa et al., 1993), sra encodes a calcipressin (a protein that inhibits calcineurin serine/threonine phosphatases) (Chang et al., 2003), and gish encodes a putative kinase (Hummel et al., 2002). Although no STM discovery studies for these genes have been independently replicated to date, internal replicates permitted meta-analysis of all genes except gish (Figures S1–S4; Figure 3). Lesions in these genes produced small-to-moderate STM impairments: Pka-R1 = −25%; Pp1-87B = −30%; gish = −36%; S6KII = −37%; and sra = −51%. Interestingly, none of these loss-of-function mutations completely abolish STM. Including Pka-R1, three of these genes have been characterized in the context of the Rut pathway; sra is thought to interact with the cAMP/PKA pathway (Chang et al., 2003), while epistasis analysis showed that gish STM function is independent of rut (Tan et al., 2010).

STM relies on serotonin receptors

Serotonin (5-hydroxytryptamine; 5-HT) signalling influences numerous Drosophila behaviours, including sleep (Nichols, 2007; Yuan et al., 2006), courtship (Pooryasin and Fiala, 2015), place learning (Sitaraman et al., 2008) and aversive olfactory conditioning. Flies express five serotonin receptors: 5HT1A, 5HT1B, 5HT2A, 5HT2B and 5HT7 (Gasque et al., 2013; Johnson et al., 2011; Majeed et al., 2016). Loss-of-function mutants for three of these receptors (5HT1B, 5HT2A, and 5HT7) have been analyzed by T-maze olfactory conditioning, but no replication studies have been reported. All three mutants exhibited at least moderate learning impairments: a 5HT1B hypomorph impaired STM by −75%, a 5-HT2A lesion reduced STM by −52% and a hypomorphic 5-HT7 mutation decreased STM by −38% (Johnson et al., 2011; Nichols, 2007)(Figure 3).

STM-relevant genes involved in nucleic-acid function

Three STM genes are associated with nucleic acid function: Adh transcription factor 1 (Adf1); polyglutamine tract-binding protein 1 (PQBP1); and mushroom body miniature (mbm). Adf1 is widely expressed during development (DeZazzo et al., 2000), and has a role in synaptic bouton formation in the larval neuromuscular junction (Timmerman et al., 2013).
**PQBP1** encodes a polyglutamine tract-binding protein that acts as a transcriptional repressor (Okazawa et al., 2002; Waragai et al., 1999). **Mbm** contains a zinc finger motif—suggesting nucleic acid binding function (Raabe et al., 2004) and is necessary for neuroblast ribosomal biogenesis (Hovhanyan et al., 2014). The effects of lesions in these genes on STM are relatively modest, and have not been independently replicated: **Adf1** = –21%; **PQBP1** = –20%; and **mbm** = –33% (Figures S5–7) (de Belle and Heisenberg, 1996; DeZazzo et al., 2000; Tamura et al., 2010).

**Additional intracellular-signaling genes**

Five additional intracellular signalling STM genes have been reported: 14-3-3ζ; **Synapsin (Syn); tribbles (trbl); arouser (aru);** and DNA fragmentation factor-related protein 2 (Drep2). 14-3-3ζ is preferentially expressed in the MB; loss-of-function alleles produce an STM reduction of –26% STM (Figure S8) (Philip et al., 2001; Skoulakis and Davis, 1996). **Syn** is a conserved presynaptic phosphoprotein, which among other functions, regulates vesicle recruitment to the readily-releasable pool (Hosaka et al., 1999; Rizzoli and Betz, 2005); meta-analysis of a study with nine STM loss-of-function experiments showed an STM reduction of –26% (Figure 3) (Godenschwege et al., 2004). A **trbl** lesion uniquely enhanced STM by +20% (Figure S9) (LaFerriere et al., 2008). Hypomorphic **aru** variants have mildly impaired performance during olfactory conditioning, eliciting an STM reduction of only –14% (Figure S10) (LaFerriere et al., 2011). **Drep2** is a synaptic protein expressed in the *Drosophila* CNS. **Drep2** expression is especially pronounced at the postsynaptic densities of synapses between projection neurons and Kenyon cells (Andlauer et al., 2014); two **Drep2** deletion alleles decrease olfactory conditioning performance by –40% (Figure S11)(Andlauer et al., 2014). To date, none of the effects of these five genes on STM have been replicated in an independent follow-up study.

**Extracellular-signaling STM genes**

Four STM genes were identified with extracellular-signaling functions: **NMDA receptor 1 (Nmdar1); Fasciclin 2 (Fas2); scab (scb); and amnesiac (amn).** Of these, only findings on **amn** have been replicated independently. **Nmdar1** encodes a subunit of the NMDA receptor (a heteromeric glutamate-gated cation channel), and is weakly expressed throughout the adult fly brain (Xia et al., 2005). An **Nmdar1** hypomorphic mutation mildly disrupts olfactory learning by –28% (Figure S12)(Xia et al., 2005). **Fas2** is expressed in the MB and is involved in axon guidance and cell adhesion during development (Lin and Goodman, 1994; Schuster et al., 1996), and may facilitate dopaminergic input (Cheng et al., 2001). The meta-analysis of 19 experiments from a study on **Fas2** loss-of-function mutants revealed an overall STM impairment of –26% (Figure S13). **scb** is a plasma-membrane α-integrin involved in cell adhesion, and is hypothesized to remodel synapses.
during learning (Grotewiel et al., 1998). The meta-analysis of scb loss-of-function data from 23 experiments found in two studies (Beck et al., 2000; Grotewiel et al., 1998) indicated an overall STM reduction of –49% (Figure S14). Amn is a putative neuropeptide gene that is expressed in two MB-extrinsic dorsal-paired-medial neurons (Waddell et al., 2000). Amn function is required for STM formation; meta-analysis of four experiments in three studies found an overall STM impairment of –30% (DeZazzo et al., 1999; Folkers et al., 1993; Tully and Quinn, 1985) (Figure S15).

**STM genes with no known molecular function**

Two genes with no-known-molecular function have been implicated in STM: *ethanol sensitive with low memory (elm)*; and *no extended memory (nemy)*. Elm is predicted to encode a calcium-binding protein, and influences both ethanol sensitivity and STM (LaFerriere et al., 2008); an insertional *elm* allele reduced STM by –44% (Figure 3). Nemy is predicted to alter the transcription of neighbouring genes CG8776 and CG8772 ((Kamyshev et al., 2002)); a loss-of-function *nemy* mutation reduced STM by –17% (Kamyshev et al., 2002) (Figure 3). The STM experiments concerning both of these genes have not yet been replicated.

**Replicated findings show no evidence of publication bias**

Publication bias is the publication of only positive results and neglect of negative data (Easterbrook et al., 1991). We used a funnel-plot analysis (Egger et al., 1997) to examine whether the meta-analysed data had a SE–effect size relationship consistent with publication bias. For this, we selected genes with ≥10 iterations (N) in the meta-analysis (Egger et al., 1997; Sterne et al., 2011); this selection resulted in nine eligible genes. None of the funnel plots for each gene showed any appreciable asymmetry (Figure 10 A-I). This result is consistent with the proposal that the independently replicated STM data are unbiased.
Figure 10. Funnel plot analysis of STM genes shows no publication bias

A–I. A comparison of the standard error (a measure of precision) with the performance percent change (effect sizes from all studies) for each respective gene shows a largely symmetrical distribution around the meta-analytic summary effect size (triangular apex and vertical black line). The Egger’s regression test, which evaluates the significance of the bias, is consistent with the assumption of no publication bias for all genes plotted.
Overall heterogeneity across experiments is low
As previously discussed, the degree of variability across experiments in a meta-analysis can be described by the heterogeneity metric (Higgins et al., 2003). Heterogeneity (I²%) is closely related to reproducibility—the lower the heterogeneity, the more closely inter-study data agree. We calculated heterogeneity for all STM genes. Considering that combining different alleles may increase heterogeneity, we also conducted—where needed—subgroup analyses of individual alleles or heteroallelic combinations. Of the 23 meta-analysed genes, the majority (17) showed low overall heterogeneity (I² ≤ 50%); only two (amn and Fmr1) showed high overall heterogeneity (I² > 75%)(Table 1). We observed large differences in inter-allelic heterogeneity among the subgroups of amn, dnc, Fmr1, NF-1, rg, and rut (Table 1). By contrast, intra-allelic heterogeneity was low for all genes except for the aru8.128 allele (I² = 81.36%)(Supplementary File 2).
Table 1: Reproducibility estimates from 23 meta-analyses

Heterogeneity ($I^2$) and mean absolute difference (MAD) values calculated for short-term memory-gene meta-analyses. Heterogeneity indicates the proportion of variance not attributable to sampling error alone; MAD indicates the mean difference between replicates. A hyphen indicates the metric is not applicable to the data set.

| Gene | Inter allelic %I² | Overall %I² | Inter allelic MAD | Overall MAD |
|------|-------------------|-------------|-------------------|-------------|
| Adf1sal | 0                 | 0           | 27                | 4           |
| amn   | 88                | 88          | 24                | 24          |
| aru   | 0                 | 50          | 27                | 20          |
| DAT   | -                 | 0           | -                 | 14          |
| dnc   | 91                | 61          | 50                | 14          |
| Dop1R1| 4                 | 0           | 3                 | 6           |
| Dop2R | 0                 | 0           | 28                | 8           |
| Drep2 | 72                | 39          | 39                | 11          |
| Fas2  | 0                 | 0           | 5                 | 9           |
| Fmr1  | 84                | 84          | 46                | 46          |
| mbm   | -                 | 0           | -                 | 1           |
| Nf1   | 91                | 63          | 1                 | 18          |
| Nmdar1| 46                | 46          | 13                | 13          |
| Pka-R1| 0                 | 0           | 5                 | 5           |
| Pp1-87B| -                 | 36          | -                 | 24          |
| PQBP1 | -                 | 0           | -                 | 0           |
| rg    | 92                | 72          | 26                | 19          |
| rut   | 84                | 65          | 37                | 18          |
| S6kII | 0                 | 0           | 48                | 9           |
| scb   | 63                | 46          | 18                | 22          |
| sra   | 35                | 0           | 4                 | 15          |
| trbl  | -                 | 0           | -                 | 5           |
| 14-3-3ζ| 0                 | 0           | 17                | 3           |
Figure 11. Heterogeneity distribution in the meta-analysed data set

A. Effect size does not correlate with intra-allelic heterogeneity (Adjusted-\(R^2 = -0.016, P = 0.6169\)). Short-term memory genes are shown as bubbles, with diameters proportional to their sample size and labelled according the replication status (see Figure 3).

B. Internally replicated results show a lower heterogeneity (\(I^2\)) than those with independent replications. \(I^2\) medians for independent replicates = 9.441 (interquartile range, IQR 0 to 59.96), shared-authors = 0 (IQR 0 to 9.24), and single-article = 0 (IQR 0 to 0).
Because small changes in STM are harder to detect than large changes, we hypothesized that genes with subtle performance changes would also have poor reproducibility. To test this hypothesis, we plotted the intra-allelic heterogeneities against allelic effect sizes. We found no relationship between effect size and heterogeneity (adjusted-$R^2 = -0.016$, $P = 0.6169$, Figure 11A).

As most studies on STM genes have not been replicated, we next asked whether heterogeneity increased when data were derived from independent replications. To test this hypothesis, we grouped the heterogeneity scores according to the allele's replication status (for classification see Figure 3). As expected, genes with a higher replication status generated more heterogeneous results compared to those with a lower replication status (Figure 11B). Heterogeneity in datasets from independently replicated genes (median = 9.4) was substantially higher than both the heterogeneity of the shared-authors replicates (median = 0) and the within-study replicates (median = 0).

**Effect-size reproducibility is high**

Due to the drawbacks associated with $I^2$ (see Methods), we also calculated the MAD as an independent estimate of reproducibility; MAD is not biased by the SE. For all tiers of replication, the majority of STM genes had a low MAD <20% (see Methods). Defining high MAD as >20%, only five of the 23 meta-analyzed genes had a high overall MAD: *amn, aru, Fmr1, Pp1-87B*, and *scb* (Table 1). Similarly, for inter-allelic and intra-allelic reproducibility, only 10 and five genes had a high MAD, respectively (Table 1, Supplementary File 2). There was no relationship between intra-allelic MAD and the effect size (adjusted-$R^2 = -0.02$, $P = 0.789$), further supporting the conclusion that small effect sizes are not harder to reproduce (Figure 12A). Between the three replication categories, we observed only subtle differences between the median MAD scores (Figure 12B). Both heterogeneity and MAD measures support that there is good reproducibility in *Drosophila* STM studies.
Figure 12. Analysis of reproducibility by mean absolute difference (MAD) scores

A. Effect size does not correlate with MAD scores (Adjusted-$R^2 = -0.02058$, $P = 0.789$). Short-term memory genes are shown in bubbles that have diameters relative to their sample size, and colors depending on the replication status (gold = independent, silver = shared-author, bronze = single-study.)

B. Internally replicated results show a somewhat lower MAD score than those replicated independently. Medians of the three tiers were only slightly different, and had substantial overlap: independent = 12.63 (interquartile range, IQR 7.05 to 14.3); same-group = 6.07 (IQR 3.28 to 12.96); and single-article = 4.99 (IQR 1.34 to 10.36). PPC = performance percent change.
Figure 13. Effect sizes between original and follow-up findings are comparable

Absolute performance percent change (|PPC|) values of the earliest T-maze experiments (green) and their independent replicates (orange) were compared. Experimental data are derived from genes for which replicate experiments are available: *rut*, *dnc*, *NF1*, *Dop1R1*, *rg*, *Fmr1*, *DAT*. The replicate effect sizes are slightly larger than the original effect sizes: median |PPC| for the earliest T-maze studies = 38.12 (interquartile range, IQR 13.54 to 82.07); median |PPC| for all replicates = 57.14 (IQR 31.66 to 70.13), U = 1373.0, P = 0.09.
Independent replications have effect sizes similar to original findings

In psychological science, effect sizes decline substantially between discovery studies and follow-up articles (Open Science Collaboration, 2015b). For the seven STM genes that have been independently replicated, we compared the average effect sizes from the earliest report for each gene with the available replicate effect sizes. In total, 31 experiments from discovery studies were matched with 105 experiments from follow-up studies. Surprisingly, the later T-maze effect sizes were, on average, +19% higher than those reported in earlier publications (Figure 13). This result refutes the hypothesis of low reproducibility, and supports the idea that STM analyses in *Drosophila* are highly reproducible.
Discussion
Meta-research aspects
Recent meta-research debate has focused on the poor reproducibility of data observed in a number of fields, including the psychological sciences (Open Science Collaboration, 2015b), and preclinical neurosciences (Button et al., 2013), and how the publication of underpowered experiments is driven by incentive structures (Munafò et al., 2017; Nuzzo, 2015; Rosenthal and Rosnow, 2009). While widespread irreproducibility is clearly an important problem in many fields, our data support that this does not hold true for *Drosophila* genetic studies of olfactory STM. Although publication bias is found in other areas of neuroscience (Mohammad et al., 2016; Sena et al., 2010), funnel plot analysis indicated that STM gene effect-size distributions were unbiased. Moreover, three quantitative measures of reproducibility (heterogeneity, MAD, and effect-size decline) indicated generally high reproducibility. Thus studies in this field appear more reproducible than other areas of brain science.

There are at least two possible interpretations as to why this finding should be. One positive hypothesis would be that the *Drosophila* STM field has superior data integrity due to the low cost of experiments that allow the use of large sample sizes. Low costs should also mean that independent replication is relatively accessible to other labs, so discovery authors—operating with this knowledge—might favor waiting to publish when key results have been extensively internally replicated. Certainly, our systematic review found ample evidence of extensive internal replication, supporting this positive view. However, the second and more skeptical hypothesis points at the relative dearth of independent replication: of the 32 identified genes, only seven have been independently replicated thus far. The bulk of STM loss-of-function replications have been conducted on *dnc* and *rut* mutants, which were both originally identified in the early 1980s (Duerr and Quinn, 1982; Livingstone et al., 1984). For each non-replicated gene, this view proposes either that there is insufficient interest for any other lab to perform a replication experiment, or that an experiment was conducted but never published. This skeptical perspective holds that the replication shortage could be a cryptic form of publication bias. Indeed, our review found no refutation studies, suggesting that the field abandons—rather than refutes—irreproducible memory genes. This abandonment hypothesis would ideally be tested with pre-registered experiments, as was done in psychology (Open Science Collaboration, 2015b).

Limitations
There are two notable limitations to this study. First, a challenge for all meta-analyses is to balance data splitting and clumping to appropriately account for experimental differences. Direct replication is a noble ideal; in
practice however, all follow-up experiments are conceptual replicates to varying degrees as there are always differences between experiments. Here, we addressed experimental variation using sub-groups to examine allelic differences, while combining data from all (non-outlier) loss-of-function alleles for a gene. This approach accommodates different alleles, while yielding a single estimate for the overall impact of each gene. We observed that the literature contains little information regarding the severity of individual alleles, or the qualitative or quantitative differences between alleles; reports of quantitative measures of allelic severity (e.g. q-PCR, ELISA, immunohistochemistry analyses) were the exception (Chang et al., 2003; Goodwin et al., 1997; Grotewiel et al., 1998; Han et al., 1992; Qiu and Davis, 1993). Due to this absence, multilevel models that might be constructed to account for this variation are inaccessible (Yildizoglu et al., 2015). Despite this limitation, we view the present method as preferable to narrative review, because meta-analyses are systematic and quantitative.

Second, there remains no optimal method, to date, to quantify reproducibility. As significance testing is now deprecated (Claridge-Chang and Assam, 2016; Cumming and Calin-Jageman, 2016; Gardner and Altman, 1986; Halsey et al., 2015), assessing reproducibility with significance test results was avoided (Open Science Collaboration, 2015b). Nevertheless, the three methods used here also have limitations: heterogeneity incurs the issues described above; MAD is not standardized, so cannot be compared across experimental systems; and effect-size decline can only provide an overview. We propose that variance-type measures of reproducibility are preferable to significance-test-result methods, which inherently introduce arbitrary threshold distortion (Halsey et al., 2015; Yildizoglu et al., 2015).

**Genetic aspects**

Our meta-analyses produced estimates of the quantitative phenotypes for all STM alleles. An advantage of model-system genetics is the ability to assess two gene lesions in combination; this approach allows geneticists to determine whether such combinations are additive or epistatic. Additivity suggests that two genes function independently, while epistasis (sub-additive or super-additive effect sizes) can mean that they operate in the same pathway. The 32 STM genes could be crossed into 1,024 possible two-way combinations; of these, 164 pairs have an effect-size sum exceeding 100%. Despite this, we detected only a few studies that contained experiments on trans-allelic combinations, including *rut–dnc* (Scheunemann et al., 2012); *rut–gish* (Tan et al., 2010); and *rut–NF1* (Guo et al., 2000). Although looking for epistasis experiments was not an original goal of the review, the observation (of a systematic set of articles) suggests that integrating existing genes into pathways is largely missing from the
field. Including the oddball effect sizes (*ple* and *trbl*), the sum of absolute effect sizes for STM genes is 1309% (Figure 3). Assuming that most gene effects are reproducible, it is difficult to account for this memory over-abundance. There are at least two possible explanations. First, many genes may fall into the same pathways: for example, there could be three pathways, each accounting for ~33% of STM and comprising roughly a dozen genes. Second, it may be that the current methods of measuring STM lesions lack specificity, and that disrupting numerous neuronal processes have multifarious effects on both memory formation *per se* and its preconditions.

All STM-gene-discovery studies demonstrated that the genetic lesion(s) disrupted memory while leaving odor sensitivity, shock sensitivity, and motor function unaffected (Supplementary File 1). These controls establish that a lesion does not affect the constituent sensory–motor systems (Mihalek et al., 1997), with the aim of establishing a specific role in associative functions. Nevertheless, apart from memory, the identified STM genes are reportedly involved in numerous additional biological processes. For example, loss of *NF1* function shortens lifespan, increases sensitivity to oxidative stress (Tong et al., 2007), disrupts circadian rhythms (Williams et al., 2001), and produces excessive grooming behaviours (King et al., 2016). The classical STM genes *rut* and *dnc* each have pleiotropic effects on at least five non-memory behaviours (Chen and Ganetzky, 2012; Donlea et al., 2009; Gailey et al., 1984; Hong et al., 2008; Kiger and Salz, 1985; Kubli, 2003; McBride et al., 1999; Perrimon et al., 1986; Siegel and Hall, 1979; Tong et al., 2007; Venkatesh et al., 2001; Zhong and Wu, 2004). That all STM genes are broadly pleiotropic raises the question as to whether such factors can be considered memory genes *per se*, or should be viewed as neuronal-function genes, perhaps with selective importance to memory cells.

The classic example of a preconditional memory lesion is one that disrupts normal MB development, but is not acutely involved in the physiological changes that occur during STM formation. In addition to olfactory-avoidance controls and neuroanatomy, this confound can be addressed, in part, with temporal control of gene function/dysfunction (Dubnau et al., 2001; McGuire et al., 2001, 2003). However, even this protocol cannot differentiate between genes that have an immediate role in associative plasticity and those that are acutely essential to normal memory-cell function. If the latter type are preferentially expressed in the memory cells, they would be almost indistinguishable from core plasticity factors.

From the 32 years of 50 STM genetics papers, only one was published in the review’s most recent three years, suggesting declining efforts in this area (Supplementary Figure 16). However, the emerging circuit-analysis field
retains a considerable reliance on the classic learning-gene literature (Cohn et al., 2015; Hige et al., 2015). Nevertheless, a complete theory of memory will require the integration of evidence from system, circuit, cellular, molecular, and genetic analyses; memory genetics remains a topic of crucial importance.

**Conclusion**

This study investigated the hypothesis that *Drosophila* memory genetics has limited reproducibility. To address this question, we performed a systematic review of the *Drosophila* STM genetics field, defined a taxonomy of replication types, performed meta-analyses, and applied several reproducibility metrics. The resulting synthesis does not support the hypothesis but instead indicates that replicated STM gene discovery experiments are highly reproducible, and that the published data are unbiased. The data synthesis also revealed that, while there is extensive internal replication, the rate of independent replication is limited. Total current STM-gene lesions have an estimated sum of deleterious effects of >1000%. Assuming their effects are all reproducible, this finding suggests either that many of the genes fall into shared pathways, or that current protocols lack specificity to identify core associative plasticity factors.
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Supplementary figures

| Study      | Figure | Allele          | %PPC [95CI]     |
|------------|--------|-----------------|-----------------|
| Goodwin 1997 | 3A     | Pka-R1^{75}     | -21 [ -35, -7]  |
| Goodwin 1997 | 3A     | Pka-R1^{1104}   | -24 [ -35, -12] |
| Goodwin 1997 | 3A     | Pka-R1^{75}/Pka-R1^{1104} | -29 [ -44, -15] |

RE Model
Heterogeneity: Inter-allelic $I^2 = 0\%$
Overall $I^2 = 0\%$
Test for heterogeneity: $Q(df=2) = 0.68 (P = 0.71)$

S1. Meta-analysis of loss of function Pka-R1 alleles

Meta-analysis of Pka-R1 loss-of-function data indicates an overall effect size of -25% [95CI -32, -17] with an overall $I^2$ of 0%. PPC = performance percent change; RE = random effects. Black squares represent the mean performance percent change (PPC) for corresponding experiments; square sizes are relative to each experiment’s weight in the meta-analytic average. Data sources are indicated in the study and figure columns; alleles are indicated in their own column. The red diamond indicates the overall effect size for Pka-R1. All error bars (including diamond vertices) represent the 95% CI. This data presentation format is repeated for all other forest plots.
### S2. Meta-analysis of loss of function S6KII alleles

Meta-analysis of S6kII loss-of-function data indicates an overall effect size of -37% [95CI -52, -23] with an overall $I^2$ of 0%. FE = fixed effects; PPC = performance percent change; RE = random effects. Blue diamonds represent the effect size of the allelic subgroups of S6kII. This data presentation format is repeated for all other forest plots.

| Study     | Figure | Allele          | %PPC [95CI] |
|-----------|--------|-----------------|-------------|
| Putz 2004 | 8      | $S6kII^{a-kII}$ | -34 [-64, -5] |
| Putz 2004 | 8      | $S6kII^{a-H24}$ | -39 [-57, -1] |

**RE Model**
- Heterogeneity: $I^2 = 0$
- Test for heterogeneity: $Q(df=1) = 0.0575 (P = 0.81)$

FE Model
- Heterogeneity: Not applicable
- Test for heterogeneity: Not applicable
S3. Meta-analysis of loss of function Pp1-87B alleles

Meta-analysis of Pp1-87B loss-of-function data indicates an overall effect size of -30% [95CI -50, -10] with an overall $I^2$ of 36%. PPC = performance percent change; RE = random effects.
S4. Meta-analysis of loss of function sra alleles

Meta-analysis of sra loss-of-function data indicates an overall effect size of -51% [95CI -57, -45] with an overall I² of 0%. PPC = performance percent change; RE = random effects.
| Study       | Figure | Allele              | %PPC [95CI] |
|------------|--------|---------------------|-------------|
| DeZazzo 2000 | 1A     | $Ad^{psi(p)}$       | -17 [-33, 0] |
| DeZazzo 2000 | 1D     | $Ad^{psi(p)}$       | -17 [-31, -3] |
| DeZazzo 2000 | 3B     | $Ad^{psi(p)}$       | -24 [-38, -10] |
| DeZazzo 2000 | 3C     | $Ad^{psi(p)}$       | -19 [-38, 0] |
| RE Model    |        |                     |             |
| Heterogeneity: $I^2 = 0\%$ | Test for heterogeneity: $Q(df=3) = 0.60 (P = 0.90)$ | -19 [-27, -12] |
| FE Model    |        |                     |             |
| Heterogeneity: Not applicable | Test for heterogeneity: Not applicable | -23 [-33, -14] |
| RE Model    |        |                     |             |
| Heterogeneity: Inter-allelic $I^2 = 0\%$ | Overall $I^2 = 0\%$ | Test for heterogeneity: $Q(df=4) = 0.9781 (P = 0.91)$ | -21 [-27, -15] |

### S5. Meta-analysis of loss of function $Adf1$ alleles

Meta-analysis of $Adf1$ loss-of-function data indicates an overall effect size of -21% [95CI -27, -15] with an overall $I^2$ of 0%. FE = fixed effects; PPC = performance percent change; RE = random effects.
S6. Meta-analysis of loss of function PQBP1 alleles

Meta-analysis of PQBP1 loss-of-function data indicates an overall effect size of -20% [95CI -31, -9] with an overall $I^2$ of 0%. PPC = performance percent change; RE = random effects.
Meta-analysis of loss of function $mbm$ alleles

Meta-analysis of $mbm$ loss-of-function data indicates an overall effect size of -28% [95CI -53, -2] with an overall $I^2$ of 0%. PPC = performance percent change; RE = random effects.

### Table: Meta-analysis of loss of function $mbm$ alleles

| Study          | Figure | Allele   | %PPC [95CI] |
|----------------|--------|----------|-------------|
| De Belle 1996 | 3A     | $mbm^{N337}$ | -27 [-58, 4] |
| De Belle 1996 | 3E     | $mbm^{N337}$ | -29 [-72, 15] |

**RE Model**

Heterogeneity: Inter-allelic $F$ = Not applicable

Overall $F = 0\%$

Test for heterogeneity: $Q(df=1) = 0.0027 (P = 0.96)$

| Performance percent change |
|---------------------------|
| -100 -50 0 25             |
### S8. Meta-analysis of loss of function 14-3-3ζ alleles

Meta-analysis of 14-3-3ζ loss-of-function data indicates an overall effect size of -26% [95CI -29, -23] with an overall I² of 0%. FE = fixed effects; PPC = performance percent change; RE = random effects. The grey-coloured rows indicate the outliers that were excluded from the calculations based on a Z-score outlier filter (see Methods). This data presentation format is repeated for all other forest plots.
S9. Meta-analysis of loss of function trbl alleles

Meta-analysis of trbl loss-of-function data indicates an overall effect size of 20% [95CI 6, 33] with an overall $I^2$ of 0%. PPC = performance percent change; RE = random effects.
**S10. Meta-analysis of loss of function aru alleles**

Meta-analysis of aru loss-of-function data indicates an overall effect size of -14% [95CI -31, 2] with an overall I² of 50%. PPC = performance percent change; RE = random effects.

| Study          | Figure | Allele | %PPC [95CI]               |
|----------------|--------|--------|---------------------------|
| LaFerriere 2011 | 2      | aru^{E-28} | -34 [-60, -9]             |
| LaFerriere 2011 | 2      | aru^{E-28} | 3 [-15, 21]               |
| RE Model       |        |        | -15 [-51, 21]             |
| LaFerriere 2011 | 5B     | aru^{51S} | -22 [-57, 12]             |
| LaFerriere 2011 | 5A     | aru^{51S} | -13 [-34, 8]              |
| RE Model       |        |        | -15 [-33, 2]              |
| RE Model       |        |        | -14 [-31, 2]              |

Heterogeneity: F = 81%

Test for heterogeneity: Q(df=1) = 5.3643 (P = 0.02)

Heterogeneity: F = 0%

Test for heterogeneity: Q(df=1) = 0.2181 (P = 0.64)
S11. Meta-analysis of loss of function Drep2 alleles

Meta-analysis of Drep2 loss-of-function data indicates an overall effect size of -40% [95CI -47, -32] with an overall I² of 39%. FE = fixed effects; PPC = performance percent change; RE = random effects.
### S12. Meta-analysis of loss of function Nmdar1 alleles

Meta-analysis of Nmdar1 loss-of-function data indicates an overall effect size of -28% [95CI -40, -15] with an overall $I^2$ of 46%. FE = fixed effects; PPC = performance percent change; RE = random effects.

| Study  | Figure | Allele  | %PPC [95CI] |
|--------|--------|---------|-------------|
| Xia 2005 | 4C | Nmdar1$^{EP331}$ | -23 [-32, -14] |
|        |       | FE Model |             |
|        |       | Heterogeneity: Not applicable |             |
|        |       | Test for heterogeneity: Not applicable |             |
| Xia 2005 | 4D | Nmdar1$^{EP331}$ | -36 [-52, -20] |
|        |       | FE Model |             |
|        |       | Heterogeneity: Not applicable |             |
|        |       | Test for heterogeneity: Not applicable |             |
|        |       | RE Model |             |
|        |       | Heterogeneity: Inter-allelic $F^2 = 46\%$ |             |
|        |       | Overall $F^2 = 46\%$ |             |
|        |       | Test for heterogeneity: $Q(df=1) = 1.8441 (P = 0.18)$ |             |

Performance percent change
## S13. Meta-analysis of loss of function Fas2 alleles

Meta-analysis of Fas2 loss-of-function data indicates an overall effect size of -26% [95CI -32, -19] with an overall I² of 0%. PPC = performance percent change; RE = random effects.

### Table: Meta-analysis of Fas2 loss-of-function data

| Study      | Figure | Allele   | %PPC [95CI] |
|------------|--------|----------|-------------|
| Cheng 2001 | 5B     | Fas2<sup>2</sup> | -39 [ -67, -11 ] |
| Cheng 2001 | 5B     | Fas2<sup>2</sup> | -34 [ -63, -5 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -21 [ -62, 19 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -20 [ -37, -3 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -14 [ -37, 10 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -26 [ -54, 1 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -17 [ -62, 25 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -31 [ -90, 25 ] |
| Cheng 2001 | 5A     | Fas2<sup>2</sup> | -26 [ -59, -13 ] |

RE Model

Heterogeneity: F = 0%
Test for heterogeneity: Q(df=8) = 3.6131 (P = 0.89)

| Study      | Figure | Allele   | %PPC [95CI] |
|------------|--------|----------|-------------|
| Cheng 2001 | 5C     | Fas2<sup>2</sup> | -31 [ -50, -11 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -17 [ -44, 9 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -21 [ -45, 3 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -25 [ -57, 7 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -32 [ -66, 1 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -30 [ -69, 9 ] |
| Cheng 2001 | 6A     | Fas2<sup>2</sup> | -29 [ -77, 18 ] |
| Cheng 2001 | 5A     | Fas2<sup>2</sup> | -24 [ -44, -3 ] |

RE Model

Heterogeneity: F = 0%
Test for heterogeneity: Q(df=7) = 1.0555 (P = 0.99)

### Figure: Performance percent change

Performance percent change

-100  -50  0  25

-26 [ -32 , -19 ]

RE Model

Heterogeneity: Inter-allelic F = 0%
Overall F = 0%
Test for heterogeneity: Q(df=16) = 4.6687 (P = 1.0)
### S14. Meta-analysis of loss of function scb alleles

Meta-analysis of scb loss-of-function data indicates an overall effect size of -49% [95CI -58, -39] with an overall I² of 46%. PPC = performance percent change; RE = random effects.
### S15. Meta-analysis of loss of function amn alleles

Meta-analysis of amn loss-of-function data indicates an overall effect size of -30% [95CI -49, -11] with an overall $I^2$ of 88%. PPC = performance percent change; RE = random effects.

| Study       | Figure | Allele  | %PPC [95CI] |
|-------------|--------|---------|-------------|
| DeZazzo 1999| 1C     | amn$^{24A}$ | -26 [-42, -9] |
| DeZazzo 1999| 3C     | amn$^{Xl}$   | -63 [-85, -42] |
| Folkers 1993| 2      | amn$^{l}$   | -19 [-27, -11] |
| Tully 1985  | 10     | amn$^{P60J}$ | -18 [-31, -5] |

**Heterogeneity:**
- Inter-allelic $I = 88\%$
- Overall $I = 88\%$

**Test for heterogeneity:** $Q(df=3) = 15.5098 \ (P = 0.001)$

![Performance percent change graph]
S16. Publication dates of the 50 STM-gene articles identified by the systematic review.