

Behavioral variation and donor history in the follow-up of somatic cell nuclear transfer

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Abstract

We examined task acquisition and lateral-return behavior in a mouse somatic cell nuclear transfer series and compared selected human and macaque records. Eight mouse donors supplied fibroblasts before and after directional training, producing 19 and 21 animals eligible for behavioral assessment; four handled donors supplied 12 additional animals from later collections only. After the clones' own operant training, the retained summaries classified stable lateral returns in 2/19 pretraining-source and 8/21 posttraining-source animals. Returns matched the donor's assigned direction in 1/19 and 7/21, respectively. The equal-donor matching difference was 29.2 percentage points, alongside a 27.1-point increase in any stable side preference. These are descriptive contrasts: directional analysis was exploratory, source period was confounded with elapsed time, and trainer masking and numerical-rule timing were unverified. All 52 eligible animals were recorded as reaching criterion; undocumented session accounting prevents comparison of acquisition efficiency. An incidental care observation in 2018 prompted a separate human-record review. A repeated receiver-handling action could be counted, whereas a speech correspondence remained a broad phonetic description. Unequal observation opportunities and incomplete exposure histories limit those selected cases. The report identifies dimensions that criterion attainment alone would miss and defines comparisons needed to distinguish general repetition from donor-specific correspondence. It does not establish acquired-memory transfer or assess the accelerated-maturation procedures of the wider program.

Introduction

The practical endpoint of a cloning program is often stated as the production of a viable organism. For a program intended to shorten the interval between cell collection and functional use, that endpoint is insufficient. An animal can survive, attain an expected body size and pass a routine locomotor examination while still requiring a prolonged period of learning. Conversely, an unusual movement in an otherwise competent animal may be dismissed as an individual mannerism even when it repeatedly interferes with a trained task. These outcomes require different measurements. A production record alone supplies neither a behavioral age nor a measure of competence.

BioDome began funding the rapid-cloning program in 1992. Work continued intermittently as production difficulties, changes in personnel and competing institutional priorities allowed. The initial objective was to shorten production and somatic maturation; reproducing an individual's acquired memories was not a condition of the original program.

Developmental follow-up gradually accumulated alongside production records. Lambrecht's investigations of relatively stereotyped behavior formed one part of that follow-up, without yielding a general account of how a behavioral disposition emerges in a newly developing nervous system. Stewart assumed operational responsibility for the later comparative work. The present report concerns behavioral assessment within that longer program, rather than the technical performance of its accelerated-maturation procedures.

Published somatic cell nuclear transfer (SCNT) studies provide useful precedents for separating these questions. Nuclear transfer has produced mice and macaques, while long-term assessment of surviving cloned sheep has examined physiological health at ages far beyond the original production endpoint.[1-3] A study of cloned dogs instead compared learning and exploration, including variation among animals sharing a nuclear genotype.[4] These are different forms of follow-up. Similar performance on a task does not identify the history that produced it, and a narrow distribution among related animals does not show that a donor's learned solution has been copied. Each animal in such an assessment has a developmental and training history of its own.

The same distinction matters when discussing instinct. Stereotyped behavior can depend on inherited developmental organization without being a reproduction of a parent's or donor's experience. Individual flies, for example, can maintain a locomotor turning preference, and interventions in identified neural populations can alter the distribution of those preferences.[5] Such work makes a specific link between neural organization and behavioral variation. It gives no reason to equate the stability of one individual's movement with the inheritance of that movement. Our concern is the narrower possibility that a donor's acquired experience could be associated with a change in a source cell that subsequently biases development or action selection.

That possibility imposes a demanding mechanistic requirement. Motor learning changes neural circuits; synaptic stabilization and activity marking learned sequence boundaries have been observed during skill acquisition.[6,7] A skin-derived nucleus is removed from those circuits. Persistent chromatin states can affect nuclear reprogramming,[8,9] but cellular memory in this sense concerns prior transcriptional organization. To explain a correspondence with an acquired action, an account must also explain how relevant specificity reaches the sampled tissue and influences a new nervous system. Evidence that some chromatin states resist reprogramming does not supply either missing step.

The mouse series was planned in 2015 as a source-history and functional-follow-up study. A conspicuous receiver-handling action, noticed incidentally by a member of the care staff in 2018, subsequently prompted closer comparison of archived donor and recipient behavior. The mouse production and macaque assessments preceded that observation. We use the retained mouse summaries to describe acquisition under the administered training and the form of later returns. Within those returns, we compare stable side preference generally with agreement with the donor's assigned direction. The archival review then asks how specifically a proposed correspondence can be defined when a controlled comparison is unavailable.

Materials and methods

Scope, chronology and study status

The report combines a mouse series produced in 2015-2016, developmental macaque records assembled in 2011-2014, and a human records review undertaken after the September 2018 observation. The mouse plan was signed internally before the first cell collection. It provided for acquisition assessment and classification of return movements after task learning. The surviving plan establishes those outcome domains but does not establish when every numerical acquisition or classification rule was fixed. The rules below describe the retained summaries; their prospective specification cannot be independently verified. The direction-specific analysis presented here was developed during the later comparative review and is exploratory. Neither the plan nor the subsequent analysis was publicly registered. An analysis can use blinded observations without having been specified before those observations were collected.

The mouse series used conventional gestation and postnatal rearing. It supplies a reference for source-history questions within the wider rapid-cloning program, not a test of accelerated maturation. Historical human production and accelerated somatic development are features of the originating institutional programs; their methods and yields are outside this report. The clinical records establish the subjects' recorded provenance, but the present behavioral comparison does not independently validate those production claims. The iPSCs prefix is a retained registry designation and does not denote induced pluripotent stem cells.

Production counts, veterinary eligibility totals and donor-level behavioral counts were transcribed from the study summaries into the tables supplied here. The circulated extract lacks complete animal-by-session scores and linked gestational, foster and cage histories. We therefore use it for descriptive comparisons and make no population-level significance claim. Molecular measurements are not included: source cells, separately produced embryos and selected adult tissues did not provide a balanced longitudinal series suitable for relating a source-associated feature to the later behavior. The mechanism is addressed as a research question in the Discussion.

Donors, cell collection and training

Twelve male mice from an F2 intercross of C57BL/6J and DBA/2J founders provided source material. They came from six birth litters, with no more than two donors per litter. Eight donors underwent operant training; four underwent time-matched handling and chamber exposure. The first ear-skin biopsy was collected at 10-12 weeks of age on 11 May 2015, before chamber exposure. The second was collected from the opposite ear on 22 June. Biopsy side was balanced across the study groups. Anesthesia, analgesia and a seven-day recovery period preceded training. Collection occurred on fixed dates rather than being delayed until a donor displayed a preferred behavioral result.

Each biopsy was divided between two parallel fibroblast cultures. These preparations were technical replicates of one donor-period sample. Cells were banked at passage two; paired samples were thawed together and expanded to passage three before transfer. Passage, medium lot and the interval between thawing and transfer were matched within donor pairs. Production summaries record contamination and identity checks without retaining their methods or results in this extract. Donor-period assignments are accepted from the production ledger and have not been independently reverified here. All 24 biopsies yielded usable cultures. Both collections from trained donors supplied production; only the second collection from the handled donors did so. The handled group consequently cannot estimate the change that would have occurred between two untrained collection dates.

The eight trained donors were assigned a left or right return by a concealed balanced draw, with four donors in each direction. Magazine training and lever shaping preceded a fixed-ratio-five schedule: five lever presses produced a sucrose reward at a port on the opposite wall. During directional training, a removable guide constrained the return from the reward port to the lever to the assigned side. Five sessions with the guide, described in the donor record as criterion sessions, were followed by five without it. The circulated record does not define the donor criterion; the clone acquisition threshold below cannot be assumed to apply. Donor returns during the latter period were recorded as persistent, intermittent or ceased. These descriptive categories were not used to decide the second biopsy date.

Handled donors entered the chamber on a yoked schedule and received reward at matched times with the lever covered and no guide. This arrangement matched some aspects of handling, reward and chamber exposure. It did not match instrumental contingency or the amount and direction of movement. Training is therefore a compound change in donor experience. An observed source-period difference could reflect activity, physiological state, aging over the collection interval or cell preparation, as well as learning. Pairing samples within a donor removes between-donor sequence differences from that pair; it does not remove these temporal alternatives.

Production, rearing and eligibility

Production used fibroblast SCNT by direct nuclear injection into enucleated oocytes, following a laboratory adaptation of established mouse methods.[1,10] Oocytes were obtained from B6D2F1 females and recipient and foster dams were CD-1. The production ledger records 4,560 reconstructed oocytes and 3,210 transferred embryos. The report provides the denominators needed to follow selection into behavioral testing, but does not present a new nuclear-transfer protocol. Replication of the behavioral comparison requires a validated production method held consistent across source periods; the present outcome cannot establish the interchangeability of different reprogramming procedures.

Six calendar production blocks ran between September 2015 and March 2016. Each trained donor appeared in three blocks, with its pretraining and posttraining samples processed on common dates. Oocyte pools were divided between coded conditions, and processing order and recipient allocation alternated within day. Each surrogate received a single donor-period preparation. D01-D04 and handled lines H01-H02 were produced in the first three blocks; D05-D08 and H03-H04 in the last three. Thus paired periods shared production dates, whereas donor identity was not distributed across all blocks. A difference associated with a subset of donors can also be associated with its production period. The extract does not retain a prespecified production stopping rule; the resulting group sizes cannot establish one.

Pups were fostered, weaned at 21 days and group-housed where compatible. Rearing rooms contained neither donor apparatus nor donor recordings. Testing began at 12-14 weeks. Veterinary eligibility required sufficient mobility, dentition and feeding to undertake the task; it was assessed before behavioral codes were released. Four survivors were excluded for persistent locomotor impairment and two for malocclusion affecting feeding. No animal was subsequently excluded because its target behavior was absent or unusual. The lineage, birth, survival and exclusion totals are retained in Supplementary Table S2 and Figure 1.

The number of eligible clones differs between donor periods. An animal contributes one outcome, but animals from the same donor share a source history and may share preparation or rearing conditions. The six donor birth litters are not six surrogate litters or six production blocks. Those three levels must not be interchanged. Because the circulated records do not link every eligible animal to its surrogate, foster dam and cage, a reliable hierarchical analysis cannot be reconstructed.

Task and behavioral classification

The chamber measured 30 cm wide by 24 cm deep, with a lever centered on one wall and a reward port centered on the opposite wall. Left and right were fixed apparatus coordinates viewed from the lever toward the port. During donor training, a guide across the transverse midline left an opening on the assigned side. The guide constrained travel in both directions; the reported phenotype concerns the return from reward port to lever. Clone testing used the open chamber throughout. A lateral return was defined by a crossing of the transverse midline at least 4 cm from the direct port-to-lever axis. Figure 3 shows the scoring geometry. The definition concerns the route between two task components; it does not specify all movements within a complete learned sequence.

Each clone initially received a 20-minute unrewarded exposure. It then underwent its own magazine training, lever shaping and daily 30-minute sessions progressing to fixed-ratio-five performance. The retained acquisition criterion was at least 20 earned rewards in each of two consecutive sessions without assistance. Six further sessions supplied the classification window. The last three consecutive sessions were scored, with at least 15 codable returns required in each. All 52 eligible animals were recorded as meeting the acquisition and observation requirements. The extract does not establish whether the reported sessions to criterion began with magazine training, shaping or fixed-ratio-five performance, or how preparatory assistance was counted. These summaries cannot establish comparative acquisition efficiency.

A stable lateral return required the same side on at least two thirds of codable returns in each of the final three sessions. Recurrent returns without a stable side met the corresponding total lateral-return threshold without one side meeting the stability criterion throughout. This category does not require successive returns to alternate. Animals meeting neither definition were classified as lacking a qualifying recurrent return. Matching and opposite-direction categories were assigned only after comparing the observed stable side with the donor's allocated direction. For pretraining-source animals, matching therefore refers to a direction assigned to the donor after that source sample had been collected. Handled donors had no assigned direction, so a stable return in their clones cannot be called matched or opposite.

Overhead video was recorded at 25 frames per second. Grooming, climbing, intervention, failure to return and loss of the relevant view made an interval uncodeable. Two coders assessed the primary sessions independently and resolved disagreements before release of source-period and donor-direction codes. These

procedures reduced knowledge of the target comparison during classification. The extract does not establish whether personnel shaping or testing the clones were unaware of source period, donor identity and assigned direction. Coding blindness alone cannot exclude differential shaping, handling or cueing during behavior. The surviving aggregate extract also lacks initial coder decisions, interval-level exclusions, multiple-crossing adjudications and each session's left and right counts. It permits recomputation of the reported proportions, but not independent adjudication of the threshold classifications or a test of differential missingness between groups.

Nine posttraining-source animals classified as recurrent underwent a selected follow-up. They received three variable-ratio sessions, three restored baseline sessions, and five sessions with a changed lever housing and a reward port displaced laterally by 4 cm. The order was fixed and there was no parallel unaltered comparison group. These observations describe persistence under a sequence of conditions. Changing more than one feature and selecting animals after the behavior appears prevents attribution of a change to any particular cue.

Archival material and selection

In September 2018, occupational therapist Marion E. Keller recorded an additional replacement and second lift of a receiver while iPSCs-11 handled an unplugged desk telephone during routine care. The recipient had been produced in George J. Donnelly's separate developmental program, which concerned viability, graft development and neurological organization. After Donnelly's death in an unrelated laboratory accident and the program's later closure, routine care continued. The recipient's registered birth was in June 1993. The 2018 encounter was an assessment of an existing adult recipient with substantial communication limitations, not an observation of accelerated infant development. Earlier care records did not document systematic experience with a corded desk telephone, although they could not establish lifetime absence of such exposure.

The first recorded demonstration comprised one lift toward the head followed by replacement. Three care sessions over eleven days supplied six visible contacts with the instrument. Keller knew the recipient's clinical origin but had no access to donor provenance. An archive officer countersigned the action record, and two donor-blind reviewers confirmed its order from the complete interactions before donor provenance was released. Lambrecht did not make the observation; he had relinquished routine program coordination in 2003 and contributed to the later interpretation and manuscript. Ordinary clinical care continued independently of this review.

The donor material consisted of three complete workplace ergonomics recordings from August 1991, November 1991 and January 1992, retained with a dispatch-desk assessment. Telephone handling was incidental to their original purpose. Equipment records placed a reported hook-switch fault in November 1991, followed by instrument replacement later that month. The three recordings supplied 25 interactions in which receiver removal and subsequent handling remained visible through placement beside the head. All eligible interactions in those recordings were counted; selected clips were not used as the denominator. Source-cell collection in March 1992 followed the recorded behavior. The material reached the comparative review after the 2018 care note; its earlier recording date should not be confused with the date on which the comparison was made.

The macaque record concerned SCNT-M4, flagged during developmental assessment in March 2013. Its donor's insertion training preceded source-cell collection in 2010. The archive contained 11 cloned and 14 nonclonal macaques assessed at varying ages between 6 and 30 months in 2011-2014. It had not been assembled to measure the frequency of one object-handling sequence. The present extract contains a selected M4 comparison and apparatus history, rather than a common opportunity count for all 25 animals. We therefore retain M4 as a case description and do not use the archive totals as exposed and unexposed denominators.

Six human recipients in the historical register had attained sustained interaction sufficient for structured assessment. Five had usable recipient and donor material; one lacked a suitable donor record. After the telephone observation, the other four eligible files were reviewed for recurring behavior, a donor counterpart

recorded before cell collection and available exposure information. GIAM-27 was the additional file retained. Two regional-phonetics reviewers received the complete usable coded speech set without the recipient's identity or SCNT history, donor provenance or the inheritance hypothesis. They signed their initial classifications before provenance release, then compared coded donor clips mixed with unrelated German speakers. The complete speech set included an examiner's reference to a German accent, so prior clinical language attributions were not fully masked. The BR1 case appendix provides the full case accounts, an illustrative exchange and phonetic transcriptions. The archive review was selective and retrospective. The two retained files are not a two-in-five estimate of the frequency of inherited behavior.

Analysis and reporting

The behavioral table separates stable lateral returns, their agreement with donor assignment, recurrent returns without a stable side and absence of a qualifying return. Both pooled animal proportions and equal-donor differences are reported. For donor *i*, the matching difference is the posttraining matching count divided by its eligible count, minus the corresponding pretraining fraction. The equal-donor summary is the mean of those eight differences. It prevents a larger surviving clone group from receiving more donor weight, while retaining the survivor conditioning and shared-history limitations of the series.

We report analogous summaries for any stable side preference. Comparing those outcomes makes clear whether a matching difference also accompanies a broader increase in lateral returns. Leave-one-donor calculations describe sensitivity to the observed donor composition. They are not confidence intervals. No test treating all clones as independent donor experiences is used, and no paired binary test is presented as a remedy for the unlinked litter and production structure. Acquisition medians and selected follow-up counts are reproduced as historical summaries; their underlying session series are unavailable for distributional reanalysis.

Archival action counts are reported with the recording opportunities from which they arose. They are not combined statistically with the mouse outcomes. Speech descriptions and the M4 record remain qualitative: the speech review lacks systematic token counts and acoustics, and M4 lacks common opportunity counts across the archive. Supplementary material specifies the calculation rules and what a future prospective series would have to record.

Results

Production and selection into testing

The 3,210 transferred embryos produced 69 live-born clones. Fifty-eight survived to eight weeks and 52 met veterinary eligibility: 19 derived from pretraining cells, 21 from posttraining cells and 12 from handled-control cells. The transferred-to-live-birth yield was 2.15%; 75.4% of live-born animals entered behavioral testing. Those percentages refer to different stages and should not be used interchangeably as a measure of production success. Figure 1 shows the complete sequence of reported denominators.

Every trained donor contributed eligible animals from both collections, with two or three animals per donor-period. Pretraining-source production yielded 25 live births, 21 eight-week survivors and 19 eligible animals; posttraining-source production yielded 29, 24 and 21. The handled series yielded 15, 13 and 12. The comparison thus describes animals that survived production and were sufficiently mobile and able to feed for the task. There is no behavioral outcome for the 11 animals that died before eight weeks or the six survivors excluded on veterinary grounds.

Acquisition and lateral return behavior

Five donors retained their allocated return consistently after guide removal, two intermittently, and one ceased within three sessions. In the clones' initial unrewarded exposure, the recorded summary identified no stable five-press sequence or repeatedly expressed donor-associated return. This brief unrewarded exposure was not a validated test of retained task knowledge. All groups subsequently received shaping. Recorded median sessions to the acquisition criterion were five, five and six for pretraining, posttraining and handled sources; the corresponding recorded interquartile ranges were 4-7, 4-6 and 4-7. Because the counting origin and accounting for preparatory assistance are undocumented, these values do not support a comparison of learning rate. All 52 eligible animals were recorded as reaching criterion under the administered training.

After acquisition, the retained summaries classified eight posttraining-source animals as showing a stable lateral return, compared with two pretraining-source animals. Seven of the eight posttraining stable returns matched the donor assignment, and one was opposite; the two pretraining stable returns divided one in each direction. One additional posttraining animal showed recurrent returns without a stable side. The handled group contained one stable side preference and no animal in the recurrent, unstable-side category. Table 1 and Figure 2 retain those categories separately.

Table 1. Reported return classifications among eligible clones after their own task acquisition.

Classification	Pretraining source	Posttraining source	Handled source
Stable return matching donor assignment	1	7	Not defined
Stable return opposite donor assignment	1	1	Not defined
Stable return without donor assignment	Not applicable	Not applicable	1
Recurrent return without a stable side	0	1	0
No qualifying recurrent return	17	12	11
Total eligible	19	21	12

The pooled difference in matching proportions was 28.1 percentage points: 33.3% after training compared with 5.3% before it. Giving each donor equal weight produced a difference of 29.2 percentage points. The corresponding pooled difference for any stable lateral return was 27.6 percentage points, with an equal-donor difference of 27.1 percentage points. Most of the observed increase in matching therefore occurred alongside an increase in stable side preference generally.

Five posttraining donor lines produced a matching animal, compared with one pretraining line. The matching differences were positive in D01-D04 and D06 and zero in the other three donors. Omitting each donor in turn left the equal-donor matching difference between 23.8 and 33.3 percentage points. This range describes the eight observed contributions. It does not account for the possible dependence of donors from the same litter or for a systematic difference between collection periods. The first production group, D01-D04, supplied six matching animals among eleven posttraining clones; D05-D08 supplied one among ten. Donor composition and production timing cannot be separated in that contrast.

Four of the five donors with persistent returns and one of the two intermittent donors produced a matching posttraining clone. The donor whose return ceased produced none.

This ordering is compatible with a relation to donor behavior, but the categories contain too few donor histories to estimate such a relation. Production success also changes the number of opportunities for a line to yield at least one matching animal. Figure 2 therefore displays clone counts and denominators within donor, rather than showing positive lines alone.

Expression in the selected follow-up

Six of the nine selected posttraining animals retained recurrent lateral returns under the variable-ratio condition. In the altered chamber, four continued consistently, three initially continued and then reduced or lost the movement, and two stopped. The aggregate record does not cross-tabulate these outcomes with each animal's original matching, opposite or unstable-side category. It therefore cannot show whether donor-matched returns were more persistent than the other recurrent returns.

The movements were not uniformly bound to the original fixed-ratio requirement. Their expression could nevertheless change with continued testing and altered apparatus conditions. Because the follow-up was selected and sequential, habituation, extinction, order effects and changed spatial demands remain possible contributors. These observations do not establish an invariant stored sequence.

Correspondences in the archival records

iPSCs-11 lifted the receiver, fully replaced it on the cradle, lifted it again and brought it beside the head. The additional replacement and second lift occurred in five of six visible contacts across the three sessions. The recorded demonstration contained only one lift and replacement; the complete sequence occurred on the first contact and four of five later contacts (case appendix, A3). There was no dialing, waiting for a response or sustained communicative use during the recorded contacts. The recipient used a small repertoire of stereotyped utterances without sustained generative conversation. A recorded response of "I don't know" to a question about the action had also occurred in unrelated care exchanges; it provides no reliable account of the action's origin.

The donor recordings contained no double lift in five usable interactions before the reported equipment fault, ten in twelve during the fault period and six in eight after replacement of the desk instrument. A maintenance entry described an intermittent hook-switch failure relieved by reseating the receiver, without describing the donor's action. Persistence after replacement strengthens the historical comparison, although the records do not prove that the fault caused acquisition (case appendix, A4). The care-unit telephone was a different model, and an external operational check found ordinary cradle return. The comparison nevertheless rests on six sampled hours of the donor's work and three recipient sessions. Undocumented demonstration, previous exposure and repetitive handling remain unresolved, particularly given the recipient's long care history.

SCNT-M4 made a leftward object contact, withdrew the object and initiated another approach after direct insertion had been acquired. The donor record contained a similar sequence associated with apparatus misalignment and continuing after repair. The original faulty apparatus was unavailable. Staff overlap and incomplete exposure records prevented a clean separation of donor-specific correspondence from ordinary learning within the facility. Although the comparison resembles the receiver case in its relation to an earlier equipment difficulty, that resemblance also makes a common environmental explanation relevant. A record selected for such a history cannot estimate how often equally specific correspondences occur without it.

GIAM-27 was born in February 1994 and died at eighteen months in August 1995. Eleven sessions across fourteen weeks between March and June 1995, seven developmental assessments and four clinical interviews, supplied approximately forty-three minutes of intelligible English. Three earlier clinical notes had described unusual pronunciation before donor-provenance access. The coded review identified interdental stopping, /w/ near [v], final obstruent devoicing, posterior rhotics and recurring vowel and prosodic features. One reviewer favored West Central German with the western Palatinate first; the other favored Rhine Franconian, including Palatine and Saarland alternatives. After those classifications were locked, both ranked the donor's coded recording as the closest regional comparison. The donor had grown up near Kaiserslautern with West Palatine German as her first variety.

The preserved exchange and approximate phonetic illustrations appear in case appendix B3. No German vocabulary or comprehension was demonstrated. The descriptions remain qualitative: systematic feature-by-token counts, acoustic measurements and interreviewer reliability estimates are unavailable.

Neither reviewer identified a precise locality, and the comparison cannot support individual-speaker identification. It is less causally specific than the ordered receiver action.

Accelerated somatic development, progressive metabolic illness, shared aspects of vocal anatomy and incomplete auditory exposure histories complicate the speech comparison. Chronological age is especially uninformative as a sole developmental match in this record. The remaining three comparable human files produced no retained correspondence under the post-discovery review criteria, but their opportunities to display comparable behaviors were unequal. Supplementary Table S4 records the availability and selection limits without converting those files into negative experimental controls.

Discussion

The retained summaries classify more posttraining-source animals as showing stable lateral returns. The direction of most of those returns agreed with the donor's allocated side. All eligible animals were recorded as reaching criterion after training, but acquisition efficiency remains unresolved. Because no group proceeded without shaping, the series also does not establish how much training was necessary. The measured difference concerns classifications of action form after training, rather than demonstrated earlier possession of the task.

A record of whether animals reached criterion would have overlooked most of the reported variation. All eligible clones met that criterion but differed in the routes repeatedly taken between reward and lever. For functional assessment, it is therefore useful to retain descriptions of how a task is performed alongside whether it is completed. In the present series a lateral return imposed no demonstrated loss of reward acquisition. In another task the same kind of additional action might increase delay, error or dependence on a particular apparatus. Those consequences have to be measured; an unusual movement is not itself evidence of impairment.

The matching contrast requires a more cautious interpretation than the general return contrast. A direction allocated during donor training creates a reference that is more specific than resemblance in activity or temperament. Pretraining samples make it possible to ask whether the same donor line already tended to produce that side preference. Yet the observed increase could still arise if later source material produced more stable turning generally, with a chance excess of matches in a small series. The eight stable posttraining returns include seven matches, but they are concentrated in five donor histories and especially in the first production group. Undocumented masking during clone training leaves differential shaping or cueing unresolved. Unverified threshold timing also prevents treating the reported classifications as a prospectively fixed endpoint, without establishing that the thresholds were chosen after inspection.

An adequate next comparison would include both collection periods from handled donors, balance production across dates and conceal source-period and direction labels during clone shaping, testing and coding. Balanced left/right reference labels should be allocated to handled donors before collection without training them to those sides. The joint outcomes would be matched stable, opposite stable, recurrent without a stable side, and no qualifying return. Their probabilities would distinguish a change in total stable returns from a change in the balance between matching and opposite returns. A signed directional excess, defined as the matching proportion minus the opposite proportion, is a useful summary but can also grow when a general increase amplifies an existing side imbalance. Interpretation therefore requires the full outcome distribution, preperiod results and contemporaneous handled sources. The analysis must follow the donor allocation and shared litter and production structure. More clones from an existing donor improve description of that donor's distribution without supplying another independently assigned experience.

Survival introduces a further problem. Source state can affect which reconstructed embryos develop, which offspring survive and which survivors can complete a behavioral test. If those filters differ between collections, the tested samples can differ even when no behavior-specific information has passed between generations of organisms. Reporting all production denominators helps expose this route of selection, but cannot recover the unobserved behavior of animals that died. The prospective question must therefore include

both survival and conditional behavioral outcomes. A change in matching confined to a severely selected surviving fraction would require a different explanation from a reproducible shift accompanied by similar production and developmental outcomes.

The sheep follow-up assessed aged survivors and reported earlier production losses.[3] The cloned-dog comparison illustrates the value of assessing variation in selected behavioral measures.[4] Neither precedent makes the present source-period contrast an inherited-memory test. Our donors differ genetically from one another, and our pre/post samples differ in age and preparation history.

The mechanistic problem is more restrictive than the behavioral one. A source cell may respond to systemic consequences of activity or stress. A persistent source-associated state might then influence reprogramming or later development. Those possibilities could account for changes in general movement, arousal or propensity to repeat an action without carrying the identity of a learned action. To explain agreement with an arbitrary left or right assignment, the proposed influence must preserve information that distinguishes the two experiences. Calling a state epigenetic does not establish that distinction. The direction assignment is useful precisely because it demands more than a general physiological consequence of training.

There are known mechanisms through which prior cellular state affects reprogramming. H3K9-associated barriers have been demonstrated in mouse SCNT.[8] H3K4-associated transcriptional memory has been examined in *Xenopus* and human nuclear-transfer material, with experimental improvement of development after H3K4 reduction demonstrated in *Xenopus*. [9] Cell-of-origin effects in low-passage mouse induced pluripotent cells provide a related example under a different reprogramming procedure.[11] These reprogramming findings do not identify a representation of a learned movement in skin. The motor-learning studies concern changes within existing neural circuitry,[6,7] whereas the present hypothesis requires a route from that experience to peripheral tissue and then back to a newly formed nervous system.

That route contains at least two experimentally separable questions. Does donor training generate a source-cell difference that can be distinguished from handling, movement and elapsed time? If so, does manipulating that difference alter later action selection without broadly impairing survival, motor function or learning? Tissue differences measured only after a clone has learned the task cannot establish that they preceded its behavior. Likewise, embryos produced from a stored aliquot years later cannot be treated as earlier observations of an adult animal already tested. Testing this route requires prospectively linked samples and the comparisons in Figure 4.

Instinct does not provide a shortcut through this problem. Inherited developmental organization can produce reliable species-typical responses, while stochastic development and experience generate variation among individuals. Work on fly locomotor individuality shows that stable directional differences can be studied experimentally without assuming that one individual's chosen direction was copied into another.[5] For the present program, the unresolved issue is how particular developmental dispositions would interact with an accelerated somatic trajectory and subsequent learning. The conventionally reared mice cannot answer that question for accelerated human development. They can help identify which behavioral dimensions should be measured separately when such records are assessed.

The archival cases pose a related question at a lower level of experimental control. The receiver record is unusual because the proposed correspondence includes an apparently unnecessary intermediate action and a donor history in which that action had a plausible practical origin. It supplies a candidate for a more discriminating assay than an overall impression of similarity. Nevertheless, selection followed the recipient observation, and the exposure history remains incomplete. The sequence may be more specific than a general mannerism while still arising through an unrecorded ordinary route. Keller did not know the donor provenance when she recorded the action. This limits one route of observer influence but does not reconstruct missing years of care.

GIAM-27 demonstrates why correspondences should not be pooled merely because they appear in related archives. A pronunciation pattern is distributed over articulatory gestures and linguistic exposure. The

qualitative feature descriptions make the regional judgment more inspectable, but neither the two transcriptions nor reviewer agreement excludes shared anatomy, illness or unrecorded exposure. Treating it as equivalent to a countable extra receiver lift would increase apparent replication by changing the phenotype. A donor-specific test would require systematic blinded transcription, acoustic feature definitions, an appropriate comparison set and a more complete exposure history. The geographical label alone cannot supply that test.

For rapid-cloning work, the temporal distinction between somatic development and learning remains central. Faster growth does not supply the opportunities, feedback and social interaction through which the recipient acquires competence. Even a reproducible source-associated tendency would not eliminate that requirement. There is a substantial difference between changing the probability of one action during later learning and producing an organism already able to perform the donor's occupation. Later commercial interest in preserving acquired skills should therefore remain a separate proposal. It cannot be entered retrospectively as the purpose of the original grant or used as an interpretation of these results.

The next mouse series should be judged by a narrow, declared outcome: whether an independently repeated source-period contrast tracks assigned donor experience after accounting for general return behavior and contemporaneous source changes. All assigned donors, production losses and assessed clones must remain visible in the analysis. If the matching contrast fails to recur in a sufficiently precise, prospectively specified replication while general motor repetition changes, the experience-specific account should be abandoned in favor of the broader developmental question. If it recurs, reversing action order while matching movement frequency and reward would provide a further test of what, if anything, has been retained.

Ethics, circulation and access

The animal series was conducted under the centre's animal-use oversight, with veterinary removal criteria applied independently of behavioral outcome. This restricted report does not reproduce an approval number or claim external ethical certification. Historical human production was undertaken under separate programs. The records review involved existing material and no new nuclear-transfer procedure or experimental intervention on a human recipient.

Access to identifiable clinical and donor material was restricted to the review group. Available donor permissions and care-record access decisions did not establish consent for unrestricted public release of all human audiovisual material. The historical production files also did not permit this group to determine that the originating programs met the standards that would be required for a new study. These unresolved issues limit circulation and reuse. De-identification alone is not treated as permission for public clinical publication, and inclusion here does not endorse the original production programs.

Funding and competing interests

BioDome funded the rapid-cloning program from its early stages and provided the facilities, records and personnel for this follow-up. It retains control of source lines and production information. The possibility of reducing future training requirements represents a commercial interest in subsequent research, not a demonstrated capability of the present program. Company control of materials and access limits independent examination of the report.

Author contributions

Lambrecht developed the comparative interpretation and wrote the manuscript with Stewart, who directed the mouse behavioral study. Meijer supervised source-cell preparation and production documentation. Van Tiel oversaw veterinary eligibility and developmental records. Falk reviewed the analysis and the scope of proposed molecular work. Pike reconstructed the clinical and exposure histories. Voss provided institutional resources and coordinated access. All authors reviewed the report. Keller made the incidental receiver observation and maintained the contemporaneous care record; she did not direct the later comparative analysis.

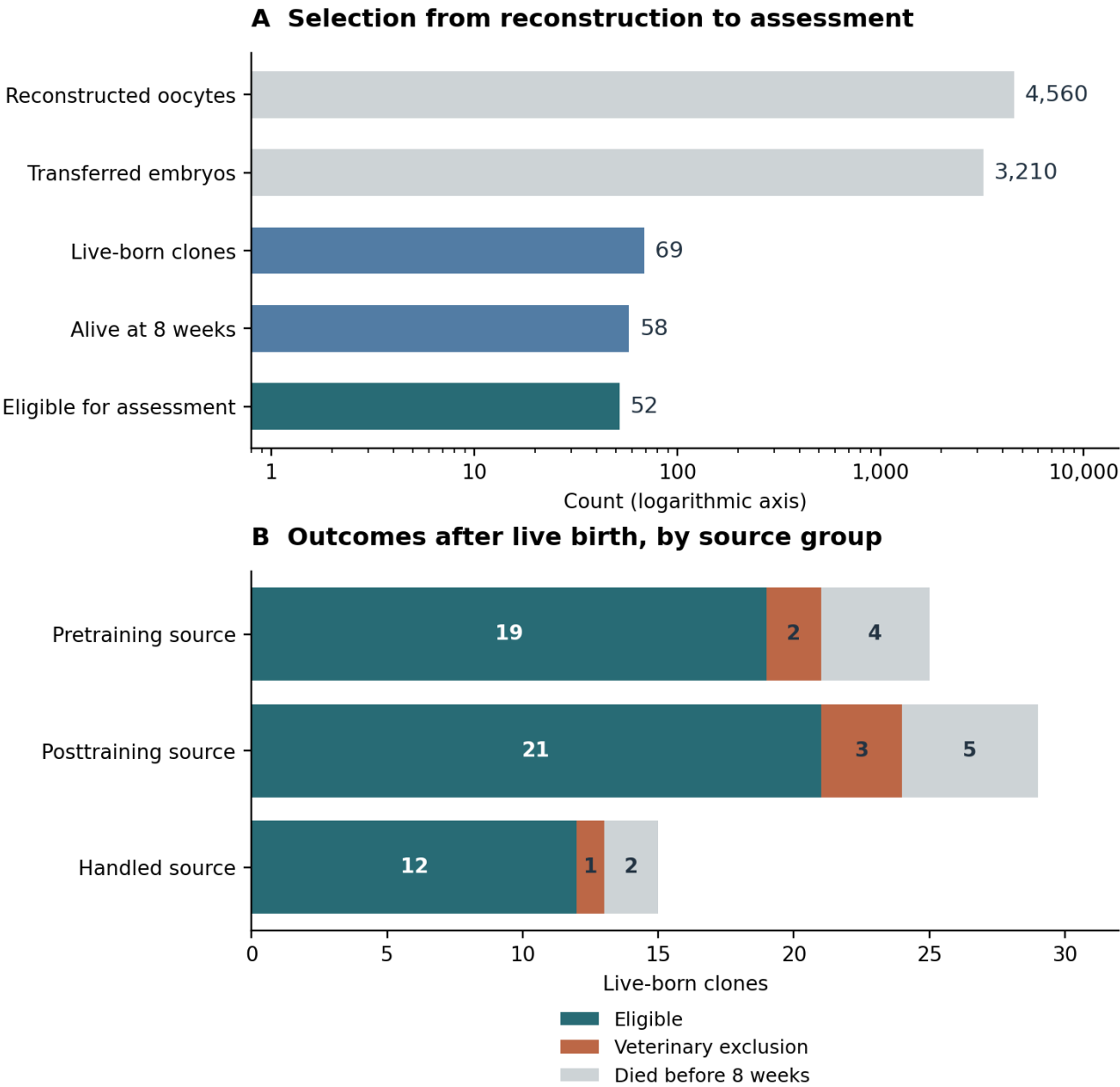
Data availability

Supplementary Tables S2 and S3 contain the aggregate production and behavioral counts used for the numerical figures; accompanying CSV files reproduce those displayed counts. The calculation script reproduces their totals, proportions and equal-donor summaries. Session-level coding, raw audiovisual records and linked rearing histories are not supplied. The available package therefore supports an arithmetic audit of the reported counts, not independent validation of the underlying observations. No public sequencing accession or molecular dataset is claimed.

References

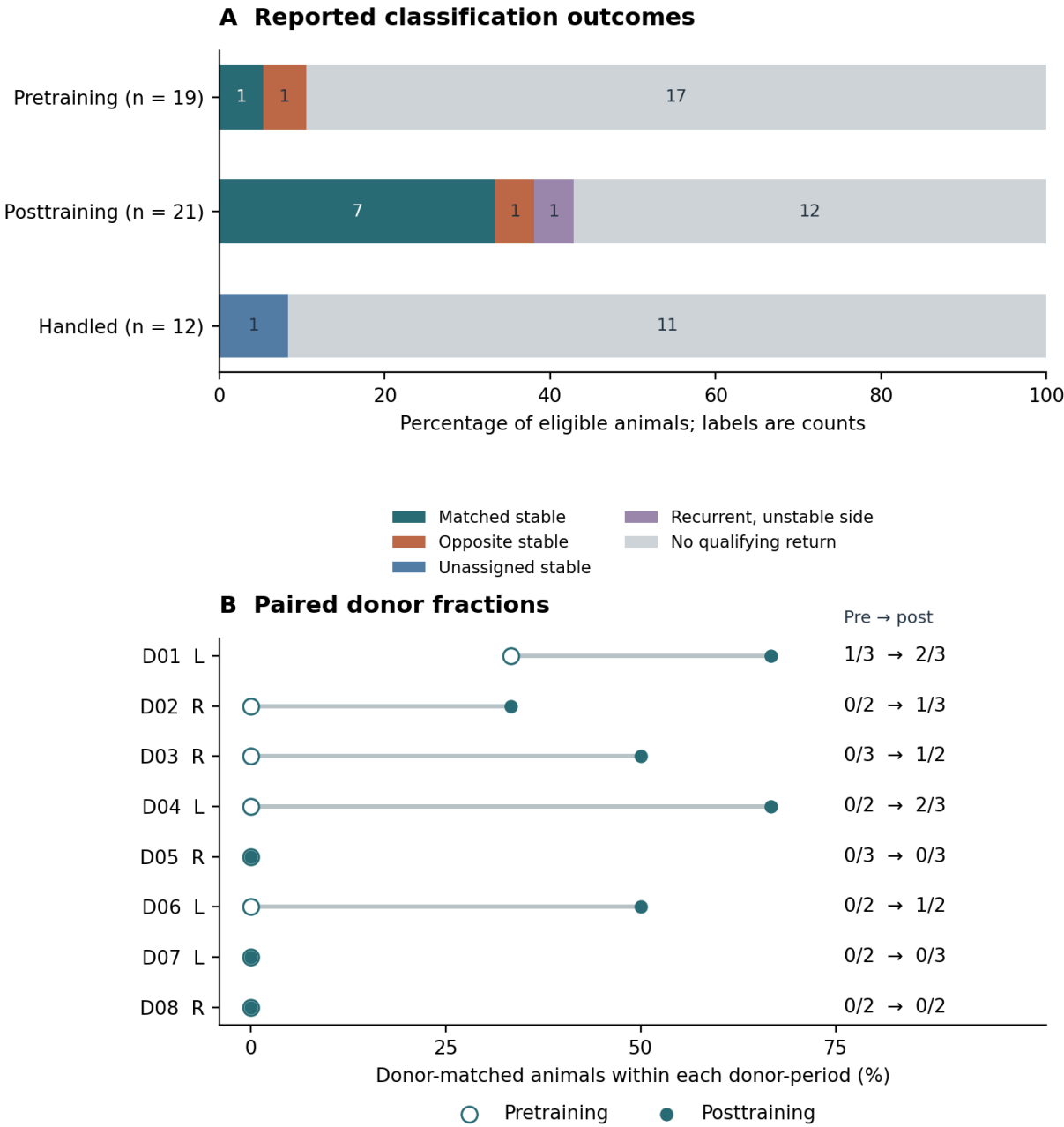
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Figure 1. Production denominators and selection into behavioral assessment



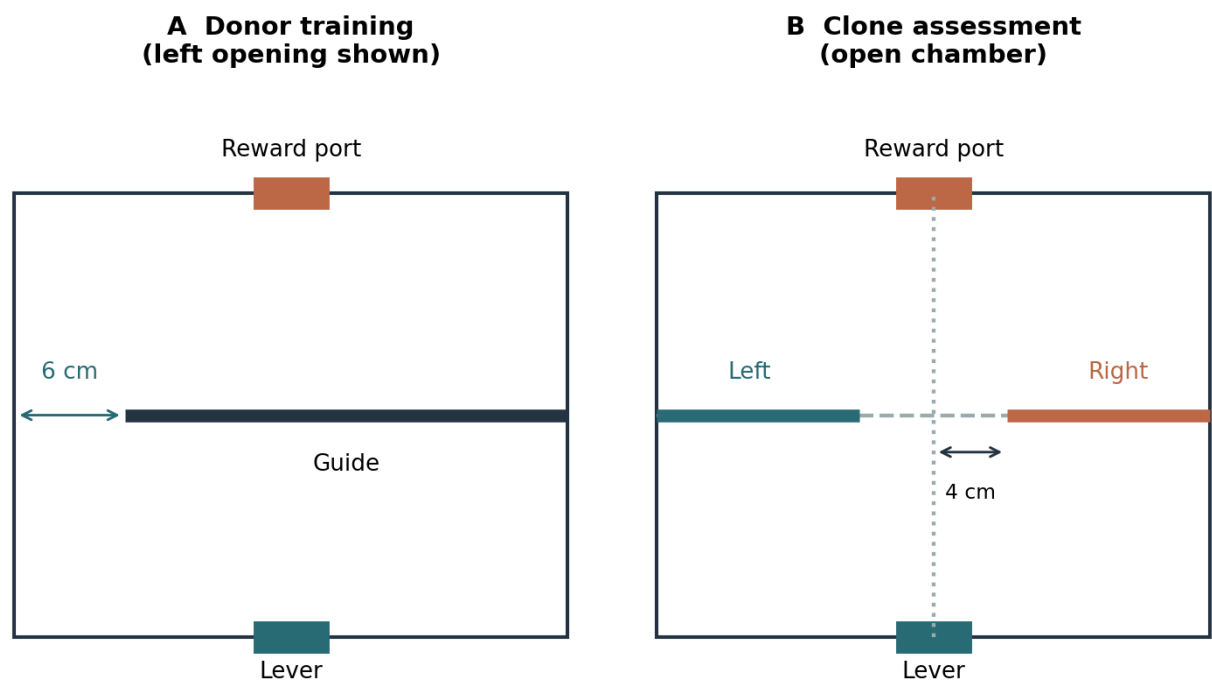
(A) Counts at successive production and follow-up stages. The horizontal axis is logarithmic; bar lengths must not be read as linear fractions of the initial cohort. (B) Outcomes among live-born clones, separated by source group. The six veterinary exclusions occurred among eight-week survivors. Counts describe this production series, with no inferred outcome for animals that did not enter behavioral assessment. Source: Supplementary Table S2.

Figure 2. Reported return classifications after the clones' own task acquisition



(A) Reported classification distributions among the 52 eligible animals. Bars show percentages; embedded labels give animal counts. Matching is undefined for handled donors because they received no directional assignment. The recurrent, unstable-side category does not establish successive alternation. (B) Matching proportions for each of the eight trained donor histories, with numerator/denominator labels. Open circles denote pretraining source; filled circles denote posttraining source. A filled point inside an open circle indicates equal proportions. Lines connect observations from the same donor, not individual clones. No error bars are shown because linked litter and rearing information needed for a defensible uncertainty model is unavailable. These plots reproduce aggregate classifications; the extract does not permit independent threshold adjudication. Source: Table 1 and Supplementary Table S3.

Figure 3. Schematic of the reported lateral-return geometry



Schematic: 30 cm wide × 24 cm deep. No observed trajectories shown.

(A) Donor-training chamber with a left-side opening shown; assigned side was balanced across donors. (B) Open chamber used for clone assessment. Colored sections mark scoring regions at least 4 cm from the direct port-to-lever axis; they are not physical barriers. Under the reported rule, a stable side required at least two thirds of codable returns on that side in each of three consecutive sessions, with at least 15 codable returns per session. This schematic illustrates the reported geometry; it is not a dated prespecified plan, a reconstructed trajectory or an image of experimental equipment.

Figure 4. Comparisons required in a prospective replication

Prospective comparison required to test specificity

Comparison	Specificity prediction	Alternative pattern
Trained and handled pre/post sources; balanced reference-side labels	Joint outcomes shift toward assigned-side matches; assess matched and opposite together	General stable returns rise; pre-existing side imbalance or source-period change
Balanced donor direction; assessment after apparatus rotation	Effect follows a defined frame of reference across new donors	Room or apparatus cues account for side preference
Same actions and rewards; different donor action order	Later expression follows assigned order beyond action frequency	Repetition or laterality changes without reliable order correspondence
Candidate source-state intervention; separate pre-task tissue cohort	Specific behavior changes without broad loss of health or learning	Effect accompanies general developmental or motor impairment

Design predictions only. None of these comparisons is reported as completed.

Predictions distinguish donor-specific correspondence from general repetition, source-period change and environmental control. Matching and opposite-direction outcomes must be considered jointly, with total stable-return probability retained. A signed excess of matching over opposite returns alone can increase when a general change amplifies an existing side imbalance. Reference-side labels for handled donors would be allocated before collection without directional training. Clone trainers and handlers, as well as video coders, must remain masked to source condition and donor or reference direction through clone training and assessment; scoring-stage masking alone cannot remove differential shaping. These are proposed comparisons, not results. The source-state intervention requires an independently reproduced behavioral contrast and controls for survival, development and task acquisition. New donors, full production denominators and linked rearing records are required throughout.

BR1 supplementary material

Fictional research report. Aggregate values are author-constructed story data.

Table S1. Program chronology and evidence availability

Period	Program or observation	Role in report
1991-1992	Donor workplace recordings; source biopsy in March 1992	Recorded before source collection; compared only after the 2018 observation
1992 onward	Company funding for rapid cloning; intermittent work over more than twenty years	Original objective: production and somatic maturation
1993	Registered birth of iPSCs-11 in a separate developmental program	Existing recipient; no planned memory experiment
1994-1995	GIAM-27 clinical record and speech recordings	Historical material reviewed in 2018-2019
2000-2007	No dedicated behavioral experimental budget; care and records continued	Interruption is not continuous behavioral experimentation
2003 / 2008	Lambrecht leaves routine coordination / Stewart assumes later operational direction	Conceptual work separated from later day-to-day responsibility
2011-2014	Macaque developmental archive; M4 flagged in 2013	Predates telephone observation
2015-2016	Paired mouse source collections, production and behavioral assessment	Planned outcome domains; later directional reanalysis exploratory
September 2018	Keller records receiver action in routine care	Incidental observation followed by donor-record comparison
2018-2019	Human archive review and comparative interpretation	Cannot prospectively motivate earlier studies
2019	Restricted technical report BR1	No public journal-publication claim

Table S2. Production and eligibility by donor-period

Reconstructed oocytes, transferred embryos, live births, eight-week survivors and veterinary exclusions are successive production/assessment stages. Each row identifies a source line, not an independently assigned behavioral history. Counts exclude molecular-only production. Individual gestational/foster/cage links are unavailable.

Donor	Period	Reconstructed	Transferred	Born	8 weeks	Excluded	Eligible
D01	pre	238	170	4	3	0	3
D01	post	243	174	4	3	0	3
D02	pre	201	140	2	2	0	2
D02	post	214	148	4	3	0	3
D03	pre	225	160	3	3	0	3
D03	post	216	150	3	3	1	2
D04	pre	207	146	3	3	1	2
D04	post	232	164	4	3	0	3
D05	pre	244	172	4	3	0	3
D05	post	248	174	4	4	1	3
D06	pre	196	138	2	2	0	2

Donor	Period	Reconstructed	Transferred	Born	8 weeks	Excluded	Eligible
D06	post	204	142	3	2	0	2
D07	pre	218	152	3	2	0	2
D07	post	238	166	4	4	1	3
D08	pre	211	150	4	3	1	2
D08	post	219	154	3	2	0	2
H01	handled	251	178	4	3	0	3
H02	handled	269	190	4	4	1	3
H03	handled	247	174	4	3	0	3
H04	handled	239	168	3	3	0	3

Total: 4,560 reconstructed; 3,210 transferred; 69 born; 58 eight-week survivors; six exclusions; 52 eligible. Malocclusion exclusions occurred in D04-pre and D07-post. Locomotor exclusions occurred in D08-pre, D03-post, D05-post and H02. D01-D04 and H01-H02 were processed in B1-B3; D05-D08 and H03-H04 in B4-B6. Paired sources shared production dates. Donor birth litters, surrogate litters, foster litters and cages are distinct levels.

Table S3. Behavioral counts by trained donor

P: persistent donor return after guide removal; I: intermittent; C: ceased. M: stable matched return; O: stable opposite return; U: recurrent return without a stable side. Remaining eligible animals had no qualifying recurrent return. Preperiod U counts were zero throughout. Categories are reported classifications, not independently rescored observations.

Donor	Side	Donor return	Pre n	Pre M	Pre O	Post n	Post M	Post O	Post U
D01	L	P	3	1	0	3	2	0	0
D02	R	P	2	0	0	3	1	0	0
D03	R	P	3	0	0	2	1	0	0
D04	L	P	2	0	0	3	2	0	0
D05	R	P	3	0	0	3	0	1	0
D06	L	I	2	0	1	2	1	0	0
D07	L	I	2	0	0	3	0	0	1
D08	R	C	2	0	0	2	0	0	0

All four handled lines supplied three eligible animals. H03 contained one stable side preference; the other eleven had no qualifying recurrent return. Handled matching is undefined.

Table S4. Archival availability and interpretation

Material	Available comparison	Missing information	Permitted use
iPSCs-11	Recipient 5/6 contacts; donor 0/5 before fault, 10/12 during fault, 6/8 after replacement	Lifetime exposure; uniform comparison actions; unsampled calls	Selected correspondence
GIAM-27	11 sessions over 14 weeks; about 43 minutes; qualitative phonetic features, two approximate transcriptions and signed regional classifications (case appendix B)	Systematic token counts, acoustic measurements, interviewer reliability, complete auditory exposure	Qualitative clinical record; no precise locality or speaker identification
Other human files	Six sustained-responsive recipients; five comparable; two retained	Equal opportunities and pre-discovery selection criteria	Selection accounting, not prevalence/negative controls
SCNT-M4	Selected insertion sequence and donor apparatus history	Original apparatus; complete exposure; uniform archive assessment	Selected qualitative comparison
Other macaque records	11 cloned and 14 nonclonal animals	Common sequence definition and opportunities across all 25	Archive coverage only

Supplementary analysis S1. Reproducible aggregate calculations

For each donor, subtract the pretraining matching proportion from the posttraining matching proportion. In donor order D01-D08, these differences are 1/3, 1/3, 1/2, 2/3, 0, 1/2, 0, 0. Their sum is 7/3 and their mean is 7/24, or 29.17 percentage points. The pooled difference is 7/21 minus 1/19, or 28.07 percentage points.

For any stable side, include both matched and opposite stable returns. The corresponding equal-donor difference is 13/48, or 27.08 percentage points; the pooled difference is 8/21 minus 2/19, or 27.57 percentage points. Recurrent, unstable-side animals are excluded from stable-side counts. These two outcomes use the same animals and are not independent replications.

Leaving out one donor at a time gives equal-donor matching differences of 28.57, 28.57, 26.19, 23.81, 33.33, 26.19, 33.33, 33.33 percentage points. This is a composition check, not a confidence interval, a permutation test or a repair for shared production and litter histories. No inferential P value is assigned to these aggregate contrasts.

The supplied script checks that each source-period's behavioral denominator equals its eligible production count and that survival minus veterinary exclusions equals eligibility. It does not verify source identity, video coding, missingness or biological causation. No individual-level observations are reconstructed from the aggregate counts.

The recorded acquisition summaries are medians of five, five and six sessions for pretraining, posttraining and handled sources, with interquartile ranges of 4-7, 4-6 and 4-7. The aggregate extract does not preserve a complete session series, an independently auditable acquisition clock or an assistance log. These numbers therefore remain historical summaries rather than a reanalysis of learning rate. The reported scoring and acquisition thresholds do not by themselves establish that every numerical rule was fixed in a dated protocol before observation; the circulated material does not supply that full threshold history. Figure 3 is a schematic of the reported geometry, not proof of prespecification.

Supplementary design S2. Requirements for a new study

A prospective replication should sample both trained and handled donors before and after a fixed interval. Assignment to training condition and directional experience should be recorded before collection, with donor birth litter used in allocation and analysis. Source pairs should be processed together, with all conditions distributed across production dates. Surrogate, foster and cage identifiers must remain linked to each animal through assessment. The number of attempted transfers should be fixed independently of behavioral outcomes.

Predefine stable side preference, donor matching, recurrent returns without a stable side and acquisition as distinct endpoints. Preserve left, right, direct and uncodeable return counts for each session, together with coder decisions before adjudication. Retain a dated version of the complete scoring and acquisition rules before clone training or assessment begins, and record any subsequent amendment with its timing and reason. Describe initial exposure without interpreting failure to perform an unfamiliar task as evidence of absent memory. Report developmental and production outcomes for the whole series; report behavior conditional on reaching each assessment stage.

Mask clone trainers, routine handlers and video coders to source period, donor training condition and allocated or reference direction throughout clone task acquisition and behavioral assessment. Use coded records and retain the code-release log. Blinded video coding alone cannot remove differential shaping or handling that has already occurred during clone training. The historical extract does not establish complete trainer and handler masking, so this requirement is prospective.

Define the acquisition clock before training: specify its starting event, whether magazine training and lever shaping count toward the reported session total, the allowed session duration and schedule, the progression to fixed-ratio-five, and the stopping rule. Predefine what counts as assistance, standardize permissible prompting and shaping, and record assistance and rewards for each session. Apply the same rules across groups. Preserve complete session-level records so that a median is not interpreted without knowing which learning opportunities it counts.

Allocate balanced left/right reference labels to handled donors before collection without training them to those sides. Analyze the joint outcomes of matched stable, opposite stable, recurrent without a stable side and no qualifying return. For each donor-period, let M and O be the matched and opposite proportions: $S = M + O$ measures total stable returns and $D = M - O$ measures signed directional excess. Report S and D as complementary summaries, not independent tests. D can increase when a general rise amplifies a pre-existing side imbalance. Interpret the full outcome probabilities with preperiod results and paired handled sources, using a donor-level period-by-training-by-assigned-side comparison justified by the actual randomization and dependence structure. A future analysis plan must define that comparison before source labels are released; it cannot be inferred from the present aggregate series. Determine sample size from a specified effect and plausible between-donor variation rather than from the pooled clone count.

If a directional contrast reproduces, an action-order experiment should equate component movements, exposure duration and reward while assigning different orders. A separate cohort sampled before task exposure is needed to establish temporal precedence for candidate developmental differences. Any proposed causal intervention must include survival, growth, locomotion and ordinary task-learning controls. Behavioral suppression accompanied by general impairment cannot establish a specific mechanism.