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A Comparison of Sleep and Settle Behaviours Across Twins and Singletons at 5 Months of Age

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ABSTRACT

Twin studies are important for research on genetic and environmental influences on child development, but it is imperative to test whether findings can be generalised from twins to singletons. Since the first months of life are defined by the emergence of important sleep behaviours, we compared 451 (54.8% females) twins and 77 singletons (48.1% females) on a range of sleep, settle and crying behaviours at 5 months of age. No significant differences were found regarding duration of crying or time until settled. However, singletons were reported to wake up more frequently during nighttime than twins ($F(1, 509) = 35.10, p < 0.001, \eta_p^2 = 0.065$), suggesting that, when reported in twin studies, this measure might be slightly underestimated at a mean level in relation to singletons. In conclusion, despite the unique challenges and additional caregiver load of infant twins, there seem to be few differences between twins and singletons regarding parent-reported settle and crying behaviours in early infancy.

1 | Introduction

Twin studies are fundamental for research on the genetic and environmental contributions to individual differences in a range of abilities, behaviours and difficulties. The results of behavioural genetics studies are important both in terms of estimating heritability and for driving the search for specific genetic influences as well as for understanding the relative role of shared and nonshared environments (Austerberry et al. 2022; Polderman et al. 2015). This sparks the question of the generalizability of the results from twin studies. In order for results to be relevant to the entire population, the phenomenon studied (e.g., a psychological trait) must function similarly in both twins and singletons (i.e., non-twins). Conversely, if a trait manifests itself in fundamentally different ways in twins and singletons, it is problematic to generalise results regarding, for example, heritability. Some factors point to important

developmental aspects differing between twins and singletons already at birth. For example, twins are on average born earlier (Liu and Blair 2002), have lower average birthweight (Liu and Blair 2002), and they postnatally gain weight slower and have a smaller head circumference (Buckler and Green 2004) than singletons. However, when correcting for gestational age, one study found no difference between twins and singletons regarding the timing of reaching developmental motor milestones (Brouwer et al. 2006), and when controlling for both gestational age and birthweight, another study found no difference regarding general development at 6 and 18 months of age (Lung et al. 2009). Similarly, in childhood, no difference has been found between twins and singletons regarding problem behaviour (van den Oord et al. 1995), autistic traits (Curran et al. 2011), or neurodevelopmental outcome (e.g., mental and psychomotor development scores, and presence of moderate/severe cerebral palsy; Eras et al. 2013), suggesting that at least

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Summary

- In this study, we examined whether twins and singletons differ in parent-reported sleep, crying, and settling behaviors at five months of age.
- Parent reports compared twins and singletons, finding similar crying and settling behaviors but slightly more frequent night waking in singletons.
- Overall, twins and singletons show comparable sleep and settle behaviors, supporting the generalizability of twin study findings to singletons.

some physical and behavioural aspects of early development might be similar across twins and singletons.

One important aspect of early development is the regulation of sleep and wakefulness. Approximately 10% to 30% of children and adolescents grapple with sleep disorder symptoms, ranging from transient difficulties in falling asleep and night awakenings to more severe primary sleep disorders (Byars et al. 2012; Gaylor et al. 2005; Owens 2008; Sadeh, Raviv, and Gruber 2000; Touchette et al. 2005). Research indicates that sleep problems in infants sometimes persist into early childhood (Byars et al. 2012) and correlate with future behavioural, emotional and cognitive challenges (Dahl 1996; Friedman et al. 2009; Gertner et al. 2002; Smaldone, Honig, and Byrne 2007). In earlier twin studies, high heritability has been found for parent-rated day/night sleep ratio at 6 months of age (Dionne et al. 2011), and moderate heritability has been found for parent-rated sleep duration and night awakenings in 15- to 18-month-olds (Brescianini et al. 2011; Fisher et al. 2012), suggesting that genetics play an important role in sleep-related behaviours early in life. A recent meta-analysis of all infant twin studies reported a pooled heritability for sleep functions of 35%, a shared environment estimate of 45% and a nonshared environment estimate of 20% (Austerberry et al. 2022). Twin studies of sleep have also been valuable in older children for understanding the role of sleep in co-occurring difficulties such as bullying and mental health (e.g., Madrid-Valero et al. 2020; Shakoor et al. 2021; Taylor et al. 2015).

However, sleep patterns in twins may not be generalizable to singletons, as caring for more than one infant at once poses higher demands for the parents and is more time-consuming than caring for a single infant. In addition, the infants may wake each other up, as it is common to let infant twins sleep together in the same bed (Damato, Brubaker, and Burant 2012). Although one study found sleep patterns to be similar in twins and singletons at 12–36 months of age (Bartlett and Witoonchart 2003), no one has examined sleep behaviours in twins versus singletons in the first year of life, and, to the best of our knowledge, no one has compared settle and crying behaviours in twins versus singletons. The first months of life marks a time of significant developmental change, when a circadian rhythm based on a day-night cycle develops (Heraghty et al. 2008). Infants gradually increase their sleep duration during the night and decrease their sleep duration during the day (Figueiredo et al. 2016), a transition believed to rely on the development of forebrain circuits and the consolidation of forebrain connections with the brainstem (Blumberg, Gall, and Todd 2014). The immense

changes happening in relation to sleep and settle behaviours in the first months of life highlights the importance of research focusing on this early period, and the need for generalizable results. Therefore, the main aim of this study was to examine the generalizability of sleep and settle behaviours across twins and singletons in early infancy by comparing mean levels of sleep behaviours and crying duration across twins and singletons. Analyses were preregistered at OSF (<https://osf.io/ts7yj/>).

2 | Methods

Both the twin sample and the singleton sample were recruited via letters sent to families identified via the Swedish Population register. Letters were sent to all families who had a child in the right age range, any exclusion were done later via parent interviews. While the twin study targeted a sample consisting of families throughout all of Sweden, the recruitment of singletons was restricted to the Uppsala county (due to the requirement of proximity to the infant laboratory). A further difference between the studies is that the twin study required no on-site physical participation, while the singleton study required families to visit the lab at least once.

2.1 | The Twin Sample

The twin sample consisted of 489 monozygotic (MZ) and same-sex dizygotic (DZ) twins (only one twin from each pair was included in this study), who were a part of a longitudinal twin study called Gut-2-Twin. Exclusion criteria for the Gut-2-Twin study were opposite-sex twin pairs, diagnosis of epilepsy, known presence of genetic syndrome related to autism, uncorrected vision or hearing impairment, very premature birth (prior to Week 34), presence of developmental or medical condition likely to affect brain development, birthweight below 1.5 kg, and infants where none of the biological parents were involved in the infant's care. After data collection, 16 infants were subsequently excluded due to twin-to-twin transfusion syndrome, 2 due to anomalies in MR/CT scan, 1 due to cerebral palsy, 1 due to meningitis and 2 due to lack of information about zygosity.

The twins have been assessed by their parents at multiple time points, here we include data on sleep and settle behaviours at 5 months of age. As most twins are born prematurely, the age of the twins has been corrected as chronological age minus the number of days between a full-term pregnancy (39 weeks; www.nichd.nih.gov) and their gestational age. Using the corrected age, we excluded participants that were either 40 days younger or older than 5 months (150 days) at time of assessment. Three infants were excluded due to being younger than 110 days, and 13 infants were excluded due to being older than 190 days. The final sample consisted of 451 infants (215 DZ and 236 MZ). Country of birth was only collected from the parent that filled in the background questionnaire (in most cases, the mother). Based on this information, 404 infants (90%) had at least one parent born in Sweden.

2.2 | The Singleton Sample

The final singleton sample consisted of 77 infants. First, it consisted of 25 infants who were a part of a longitudinal study of

autism (the Early Autism Sweden Study, EASE; www.smasys-kon.se). This overarching study includes children with and without an elevated likelihood of autism (defined as having a close family member with diagnosed autism). Here, we only included infants without an elevated likelihood of autism (i.e., no close family member with the diagnosis), meaning that they are typically developing and represents a general population of infants. In this sample, the caregivers of 18 infants answered questions about their ethnicity. For 16 infants (89%), both parents reported that their ethnicity is White. Second, we included 56 infants that were part of a cross-sectional eye tracking study at the Development and Neurodiversity Lab at Uppsala University. Sleep measures were collected from the parents of all typically developing children at 5 months of age. We excluded participants that were either 40 days younger or older than 5 months (150 days) at time of assessment. From the total singleton sample, four infants were excluded due to being older than 190 days. No infants were younger than 110 days. Country of birth was only collected from the parent that filled in the background questionnaire (in most cases, the mother). Based on this information, 42 infants (75%) had at least one parent born in Sweden. See Table 1 for descriptive statistics of demographic variables for the twin and singleton samples.

2.3 | Sleep and Settle Measures

As a measure of sleep and settle behaviours, we used data from the Sleep and Settle Questionnaire (SSQ; Matthey 2001) at 5 months of age (the same questionnaire was administered to both the twin and the singleton sample). This is a 34-item parent-report questionnaire that assesses infant sleep and settling behaviour. We extracted a subset of seven items: number of night awakenings; time until settled during the day, evening and night; and duration of crying during the day, evening and night (all items were reported as averages over the previous week).

These items were chosen as they cover important sleep and settle behaviours in early development. While the questionnaire also contained items on sleep duration, those questions were ambiguous and it was clear during preprocessing that caregivers interpreted those questions differently (i.e., some reported total sleep duration and some reported duration of single sleep periods, making it difficult to discern the actual sleep duration and to create a comparable variable). We therefore decided to exclude all items on sleep duration.

All items were open-ended questions answered by the caregivers. Number of night awakenings was reported as number of times per night the infant wakes up, the rest of the items were reported as durations. These durations were transformed to minutes and if parents reported a range (e.g., 10–30 min), the mean value was used. If a number less than zero was reported as the duration, without specifying the metric used, the answer was excluded (as it was unclear whether they replied in minutes or hours). Answers such as ‘a couple of minutes’ or ‘a few minutes’ were transformed to 2 min.

2.4 | Statistical Analyses

The primary analyses testing differences in group mean between twins and singletons were performed using ANCOVAs. Age and sex were included as covariates in all analyses. As a quality control, all analyses were additionally performed separately for MZ and DZ twins. All analyses were two-sided with a significance value corrected using a Bonferroni correction. As the number of sleep/settle variables was seven, the corrected significance value was $\alpha < 0.007$. If no statistically significant differences were found between twins and singletons, we regressed out age and sex from each sleep and settle variable and calculated Bayesian ANOVAs, to estimate the Bayes Factor. If a statistically significant difference between twins and singletons was

TABLE 1 | Descriptive statistics of demographic variables.

	Mean (SD) ^a , [min; max]		Group comparisons ^b <i>p</i> value (Cohen's <i>d</i>)
	Twins (<i>n</i> = 451)	Singletons (<i>n</i> = 77)	
<i>N</i> , females (%)	247 (54.8%)	37 (48.1%)	0.276 (0.135)
Age ^c at 5-month assessment (days)	141.8 (14.7) [110; 187]	162.7 (13.4) [123; 182]	<0.001* (1.439)
Family income ^d	5.48 (1.66) [1; 7]	5.93 (1.49) [2; 7]	0.041* (0.274)
Maternal education level ^e	3.39 (0.91) [1; 4]	3.70 (0.71) [1; 4]	0.001* (0.350)
Maternal age ^f (years)	31.79 (4.22) [20; 45]	33.62 (4.80) [23; 46]	0.002* (0.426)
Paternal age ^f (years)	33.83 (5.52) [23; 59]	35.86 (5.72) [26; 56]	0.056 (0.367)

* $p < 0.05$.

^aExcept for *N* females, which shows the frequency.

^bIndependent-samples *t*-test.

^cThe age for twins was corrected, and calculated as the difference between full term (39 weeks) and gestational age, subtracted from chronological age.

^dFamily income per month. Scale 1–7 where 1 = <20 K, 2 = 20–30 K, 3 = 30–40 K, 4 = 40–50 K, 5 = 50–60 K, 6 = 60–70 K, 7 = >70 K (SEK). These data were not collected in the EASE sample, meaning that the singleton group for this variable only consists of children from the Developmental and Neurodiversity Lab study at Uppsala University.

^eIn the twin sample, only the parent filling in the demographic questionnaire reported their education level, which in a vast majority of cases was the mother (only 17 fathers filled in the demographic questionnaire). Therefore, only maternal education level is reported.

^fFor the singletons taking part in the study at the Developmental and Neurodiversity Lab at Uppsala University, parental age was only collected for the parent visiting the lab.

found when running the ANCOVA, the analysis was repeated with family income, maternal education level, and maternal age as additional covariates (as there was a significant difference between singletons and twins regarding these variables). As only one twin from each twin pair was included in the study, there was no need to correct for relatedness in the twin sample in any of the analyses.

3 | Results

Descriptive statistics of the sleep, settle and crying measures are presented in Table 2.

After controlling for age and sex, there was a statistically significant difference between twins and singletons regarding wakeups per night ($F(1, 509) = 35.10, p < 0.001, \eta_p^2 = 0.065$), with a higher mean for singletons than twins. The difference was still statistically significant when additionally controlling for maternal age, maternal education level, and family income ($F(1, 446) = 32.04, p < 0.001, \eta_p^2 = 0.067$). Because Levene's test of equality of error variances was significant when age and sex (but not maternal age and family income) were included as covariates, we additionally regressed out age and sex from wakeups per night and ran a robust Welch test. Again, a statistically significant difference between groups was found (Welch(1, 90.003) = 26.533, $p < 0.001$).

No statistically significant difference was found between twins and singletons regarding time until settled during the day, after controlling for age and sex ($F(1, 518) = 0.181, p = 0.670$). Because Levene's test of equality of error variances was significant, we additionally regressed out age and sex from time until settled during the day and ran a robust Welch test. Again, no statistically significant difference was found (Welch(1, 112.582) = 0.179, $p = 0.673$).

There was no significant difference between twins and singletons regarding time until settled in the evening ($F(1, 512) = 0.173, p = 0.677$), and neither was there a difference regarding time until settled in the night ($F(1, 513) = 0.043, p = 0.835$). No statistically

significant difference was found between twins and singletons regarding crying duration during the day ($F(1, 512) = 0.485, p = 0.487$), and neither was there a difference regarding crying duration in the evening ($F(1, 514) = 0.097, p = 0.755$) or crying duration during the night ($F(1, 514) = 1.318, p = 0.251$). There were no differences in the above results when analysing DZ and MZ twins separately, except a small ($\eta_p^2 = 0.01$) but significant difference between DZ twins and singletons regarding crying duration during the night (see Supporting Information S1).

In order to estimate the likelihood of the null hypothesis for all non-significant analyses, we regressed out age and sex from each variable and then performed one-way Bayesian ANOVAs. The Bayes Factor (BF) for time until settled during the day was 0.037, meaning that the null hypothesis (i.e., no difference between groups) is ~27 times more likely than the alternative hypothesis (i.e., a difference between groups). The BF for time until settled in the evening was 0.038 (null hypothesis ~26 times more likely), and the BF for time until settled in the night was 0.036 (null hypothesis ~28 times more likely). The BF for crying duration during the day was 0.043 (null hypothesis ~23 times more likely), the BF for crying duration in the evening was 0.036 (null hypothesis ~28 times more likely), and the crying duration during the night was 0.059 (null hypothesis ~17 times more likely).

Because there was a statistically significant difference in the mean age of the two groups, we performed a sensitivity analysis where we excluded all twins younger than 150 days. After these exclusions, the mean age of the twin group was 160.8 days (as compared to 162.7 days in the singleton group). When running all above-mentioned ANCOVAs again (using only sex as covariate), the pattern of results remained the same (see Supporting Information S2 for full details).

As an additional sensitivity analysis, we calculated Z-scores for each variable, based on the total sample. In order to exclude extreme values, participants with Z-scores below -3 or above 3 on each variable were excluded from analyses with that variable, and all above-mentioned ANCOVAs were calculated again (with age and sex as covariates). The pattern of results remained the same (see Supporting Information S3 for full details).

TABLE 2 | Descriptive statistics of sleep and settle measures.

	Mean (SD) [min; max]	
	Twins ($n = 451$)	Singletons ($n = 77$)
Night awakenings (n)	2.04 (1.40) [0; 10]	3.27 (1.78) [0; 9]
Time until settled (minutes)		
Daytime	11.71 (9.26) [0; 60]	10.73 (8.03) [0; 60]
Evening	18.50 (15.63) [0; 90]	17.43 (16.29) [1.5; 120]
Nighttime	13.17 (14.82) [0; 90]	13.42 (16.04) [0; 90]
Crying duration (minutes)		
Daytime	25.50 (30.43) [0; 300]	18.39 (19.83) [0; 90]
Evening	15.71 (19.01) [0; 120]	13.55 (18.18) [0; 120]
Nighttime	4.83 (8.65) [0; 60]	5.82 (9.00) [0; 60]

4 | Discussion

This study aimed to examine potential differences between twins and singletons regarding sleep and settle behaviours at 5 months of age, a time defined by significant developmental changes and the emergence of the circadian rhythm (Heraghty et al. 2008). Settle and crying behaviours, defined as time until the infant settled during daytime, evening and nighttime, and average duration of crying during daytime, evening and nighttime, were not significantly different between twins and singletons, suggesting that twinning does not affect these behaviours in early infancy. These findings add to the growing body of literature showing small or non-existent differences in behaviour and development between twins and singletons (e.g., Curran et al. 2011; Eras et al. 2013; van den Oord et al. 1995), but see (Raz et al. 2016) for findings of a modest twin disadvantage on language and visual processing in preschool age: This, in turn,

renders important support for the use of twin studies in developmental research and the generalizability of twin study results to the whole population.

There was one exception to this general pattern of similar behaviour across twins and singletons. In contrast to an earlier study that found similar sleep patterns between 1- and 3-year-old twins and singletons (Bartlet and Witoonchart 2003), we found a statistically significant difference regarding number of wakeups per night, where singletons woke up more frequently than twins. This suggests that, although twins often share a bed during the first year of life (Damato, Brubaker, and Burant 2012), they do not seem to wake each other up or disrupt the sleep of each other. Rather, it might be the case that the presence of their co-twin calms them down, thereby improving their sleep. It is also possible that parents of twins sleep differently at this stage (Damato and Burant 2008), and that this affects the way they report their children's sleep. In addition, having two children may affect how the parents count the children's sleep behaviour (e.g., if one twin wakes up, the parents may wake up the co-twin as well, in order to establish a sleeping routine that is in sync between the two infants). However, we were not able to examine it further, as the questionnaire did not contain any items regarding these behaviours. Another factor that may have influenced the number of night awakenings in our samples is breastfeeding. Breastfed infants have been found to wake up more frequently during the night than formula-fed infants (Abdul Jafar et al. 2021; Weinraub et al. 2012), and studies have found that singletons are more likely than twins to be fully breastfed. For example, one study found that 4.1% of twins or triplets in Japan were fully breastfed compared to 44.7% of singletons (Yokoyama et al. 2006) and a large population-based study in Canada found that twin mothers were less likely than singleton mothers to exclusively breastfeed at hospital discharge (adjusted odds ratio 0.30; McDonald et al. 2012). While we did not have comparable data on breastfeeding practices in the singleton sample, 84% of the infants in the twin sample were reportedly breastfed at 2 months of age. This number is similar to the reported rate of breastfed infants in the general population in Sweden at this age (82.8%, based on children born in 2021, www.scb.se). However, there may have been differences between our samples with regards to breastfeeding, and this factor should be included in future studies on night awakenings. Another aspect that has been linked to a higher parent-reported number of night awakenings is co-sleeping (e.g., Volkovich et al. 2015), but in the current study we did not collect data on whether caregivers practiced co-sleeping or not. Therefore, more research is needed in order to explore whether co-sleeping and other sleep practices might impact the apparent difference in night awakenings between twins and singletons.

It is important to note that we relied on parent-reported behaviours in this study, and it is possible that the values reported by the parents might not reflect the full extent of the sleep and settle behaviours. However, an earlier study of 3 to 4-year-olds found that the correlation between parent-reports and objectively measured sleep duration was high (Sekine et al. 2002), indicating that parent-reports are useful in studies of early sleep behaviours. Nevertheless, there were significant differences between our twin sample and singleton sample in terms of maternal age, maternal education level, family income, and the age of assessment, which might have influenced how the parents

interpreted the questions. Another limitation is the generally high SES in both samples, and the current study should therefore be replicated in larger and more diverse samples, in order to discern the generalizability of our findings.

5 | Conclusions

No significant differences between twins and singletons were found regarding the time it takes for the infant to settle or the average crying duration, at any time of the day. This supports the hypothesis that settle and crying behaviours are not distinct between twins and singletons, and that findings from infant twin studies of these behaviours can be generalised to singletons. However, singletons were found to wake up more frequently at night than twins, a finding that requires further research to understand possible reasons for this difference.

Author Contributions

Charlotte Viktorsson: conceptualization, data curation, formal analysis, software, writing – original draft, writing – review and editing. **Angelica Ronald:** conceptualization, methodology, writing – review and editing. **Terje Falck-Ytter:** conceptualization, funding acquisition, resources, supervision, writing – review and editing.

Ethics Statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The analyses presented here were preregistered (<https://osf.io/ts7yj/>). Data and code will be made available upon reasonable request to the corresponding author. Note that sharing of pseudonymized personal data will require a data sharing agreement, according to Swedish and EU law.

Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/icd.2564>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.